Reference Guide for Developing and Executing *Bacillus anthracis* Sampling Plans in Indoor Settings

Version 4

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Disclaimer

This document was developed to serve as a reference document for local, state, and federal partners and contractors working within Incident Command who are tasked with sampling and analysis of *Bacillus anthracis* that has been released in or entered an indoor environment. This document serves as a means of standardizing incident response procedures by compiling, in a single volume, common accepted procedures recognized by Federal government agencies as best practices. The document is intended to be a "living" document that will be periodically revised as new methods and processes are developed and validated for use. Wherever possible, citations to locations on the web for the most current recommended methods and procedures are provided and should be referenced in the event of an actual response requirement. This document does not confer legal rights or impose legally binding requirements on any party, nor does it supersede existing practices, guidelines, or authorities of federal, state and local agencies responding to a *Bacillus anthracis* release into the environment. The use of non-mandatory language such as "may" or "should" in this document does not connote a requirement but rather indicates a preferred approach. Mention of commercial products does not constitute endorsement or recommendation of use.

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Acknowledgments

This document was prepared by an interagency workgroup known as the Validated Sampling Plan Working Group (VSPWG). This workgroup is chaired by the Department of Homeland Security (DHS), and includes technical and scientific experts from the Centers for Disease Control and Prevention (CDC), U.S. Environmental Protection Agency (EPA), Department of Defense (DoD), Federal Bureau of Investigation (FBI), National Institute for Standards and Technology (NIST), and Department of Energy (DOE) National Laboratories, including Pacific Northwest National Laboratory (PNNL) and Sandia National Laboratories (SNL).

Acronyms and Abbreviations

AAS	Aggressive Air Sampling
ACIP	Advisory Committee on Immunization Practices
AHJ	Authority Having Jurisdiction
ASTM	American Society for Testing and Materials
BAR	BioWatch Actionable Result
BI	Biological Indicator
BiSKit	Biological Sampling Kit
CBRN	Chemical, Biological, Radiological, and Nuclear
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CITAC	Cooperation on International Traceability in Analytical Chemistry
CJR	Combined Judgmental and Random
ClO2	Chlorine dioxide
COC	Chain of Custody
Ct	Cycle-threshold
DHS	Department of Homeland Security
DMP	Data Management Plan
DoD	Department of Defense
DOE	Department of Energy
DQO	Data Quality Objective
ECC	Environmental Clearance Committee
EPA	U.S. Environmental Protection Agency
ERLN	Environmental Response Laboratory Network
ESF	Emergency Support Function
EURACHEM	A Focus for Analytical Chemistry in Europe
FBI	Federal Bureau of Investigation
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
GAO	Government Accountability Office
GIS	Geographic Information System
HASP	Health and Safety Plan
HAZMAT	Hazardous Material
HEPA	High Efficiency Particulate Air
HHA	Hand-Held Assay (Based Immunoassay)
HHS	Health and Human Services
HSPD	Homeland Security Presidential Directive
HVAC	Heating, ventilation and air conditioning
IAP	Incident Action Plan
IC	Incident Commander

ICS/UC	Incident Command System/Unified Command
ICLN	Integrated Consortium of Laboratory Networks
ICS	Incident Command System
IND	Improvised Nuclear Device
ISO	International Organization for Standardization
JLC	Joint Leadership Council
JTTF	Joint Terrorism Task Force
LPM	Liter per minute
LOD	Limit of Detection
LRN	Laboratory Response Network
MARSSIM	Multi-Agency Radiation Survey and Site Investigation Manual
MCE	Mixed cellulose esterase
mL	Milliliter
mm	Millimeter
MOA	Memorandum of Agreement
MPN	Most Probable Number
NCG	Network Coordinating Group
NFPA	National Fire Protection Agency
NIMS	National Incident Management System
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute for Standards and Technology
NMAM	NIOSH Manual of Analytical Methods
NRC	National Research Council
NRF	National Response Framework
NRP	National Response Plan
NRT	National Response Team
NSPD	National Security Presidential Directive
OSHA	Occupational Safety and Health Administration
OSTP	Office of Science and Technology Policy
PCR	Polymerase Chain Reaction
PDA	Personal Data Assistant
PEP	Performance Evaluation Procedure
PNNL	Pacific Northwest National Laboratory
PPE	Personal Protective Equipment
QA	Quality Assurance
QC	Quality Control
QUAM	Quantifying Uncertainty in Analytical Measurement
RAP	Remedial Action Plan
RDD	Radiological Dispersal Device
RE	Recovery Efficiency
RV-PCR	Rapid-Viability PCR

SADA	Spatial Analysis and Decision Assistance
SD	Standard deviation
SMART	Sensitive Membrane Antigen Rapid Test
SNL	Sandia National Laboratories
T_0	Time Zero
TWG	Technical Working Group
UC	Unified Command
VSP	Visual Sample Plan
VSPWG	Validated Sampling Plan Working Group
WMD	Weapons of Mass Destruction

Section I: Introduction

1.1 Purpose

The purpose of this document is to present a reference document for environmental sampling of *Bacillus anthracis* (spores and vegetative cells), the causative agent of anthrax, during first response and remediation phases following the confirmation of contamination in a facility including large, complex buildings as well as single dwelling buildings. While this document does not address a wide area outdoor release scenario, some of the information provided in this document may be useful in developing an outdoor sampling strategy.

The document presents the tools (including approaches and methodologies) currently available that can be considered by sample planners and technical support staff operating within an Incident Command System/Unified Command (ICS/UC) when developing sampling plans. Most importantly, this document will help sample planners develop a sampling plan specific to each unique *Bacillus anthracis* (*B. anthracis*) contamination site as part of the incident and advise IC/UC decision makers (i.e., stakeholders, federal, state, local, and tribal leaders). A well-executed, site-specific sampling plan will assist decision makers to:

- Determine who may have been potentially exposed during the initial release
- Assess potential risk of exposure to responders entering the site
- Characterize the extent of the contamination
- Remediate/Decontaminate indoor sites of contamination
- Clear the facility for reoccupation or use

1.2 Background

Environmental sampling to determine the presence or absence of *B. anthracis* in indoor environments is an important tool for assessing potential risk of exposure to building occupants at the time of release and responders to the incident. Environmental sampling results can be used to confirm the presence of contamination; determine the extent of contamination; support informed decisions regarding the need for medical interventions and decontamination options; and determine the effectiveness of decontamination and when cleanup is adequate to permit re-entry into an area (OSHA 2002). However, sampling and analysis is just one of many components contributing to a hazard determination. The infectious dose of *B. anthracis* in humans by any route is not well-established, making it difficult to develop risk-based exposure limits. Therefore, sampling results, along with other data inputs (including epidemiological data, intelligence data, and modeling data), and operating parameters are used to make informed decisions regarding public health actions and environmental cleanup. As an outcome of meetings among subject matter experts during 2011, EPA and CDC recommended that “no detection of viable spores” be considered the most appropriate clearance goal.

To ensure consistent communication among various agencies during a response to a *B. anthracis* incident, this document uses the Validated Sampling Plan Working Group (VSPWG) definitions of *sampling strategy* and *sampling plan*. The *sampling strategy, sampling approach,* and *sampling plan* definitions were reached by consensus of the VSPWG in 2007.

> **Sampling strategy:** “A set of operating precepts and diagnostic tools (including sample collection methods; packaging and shipping protocols; sample recovery, extraction, and analytical methods; and statistical analysis packages, as appropriate) that are combined to answer specific hypotheses.” A sampling strategy includes the approach or combination of approaches to be used to select locations at which to collect samples and provides guidance that is informed by a decision support process. It also includes a compendium of information on relevant methods and the plan for action prescribing their use across multiple potential scenarios. (Using this definition of a *sampling strategy,* this Sampling Reference Guide document is a sampling strategy.)
>
> **Sampling approach**: “A methodology for selecting representative locations and surfaces for collecting samples.” A sampling approach provides the structure, when implemented in a sampling plan, for planners to draw conclusions from the sampling results. There are three kinds of sampling approaches discussed in this document: judgmental sampling, hotspot sampling, and combined judgmental and random (CJR) sampling.
>
> **Sampling plan**: “A documented approach for field execution that captures the specific combination of operating precepts and diagnostic tools used for a given scenario to answer a specific hypothesis.” A sampling plan is an executable plan of action addressing the sampling and analytical requirements of a specific situation and is formulated in accordance with the guidance of the sampling strategy. The sampling plan must specify the sampling approaches, methods, and analyses, as well as the number, types, and locations of samples to be collected in a given physical space (DHS 2007b).

The VSPWG intends that this reference document align with broader national response guidance, including the National Incident Management System **(**NIMS**)** and National Response Framework (NRF), which provides principles of a unified national approach for responding to a *B. anthracis* incident indoors. It is intended to be coupled with the understanding of the authority-having jurisdiction (AHJ) regarding local vulnerabilities and capabilities when developing its plans and guidance documents for response to incidents involving *B. anthracis* contamination. This guidance recognizes NIMS and ICS as an essential part of emergency response planning and response.

Specific conditions, such as the variation of *B. anthracis* characteristics (e.g., virulence, environmental persistence, and transmissibility), the uniqueness of a given scenario (e.g., mechanism of agent dispersal, exposed population characteristics, micro and macro environmental conditions), and the variety of available response resources make it infeasible to develop a template sampling plan in advance to address all *B. anthracis* incidents. However, this document describes key phases, decision points, and tools to consider when developing a site-specific sampling plan.

Section II: Response Phases, Coordination, and Roles and Responsibilities

2.1 Basics of a Response

The NRF presents the principles to provide a unified national approach for responding to an incident and provides guidance to all partners in preparing for national emergencies. The NRF is intended to strengthen, organize, and coordinate response actions across all levels. The doctrine of *tiered response* emphasizes response to an incident should be handled at the lowest jurisdictional level capable of handling the work. The NRF addresses incidents of all types, including acts of terrorism, major disasters, and other emergencies (DHS 2008). The NRF uses the same guiding NIMS and ICS principles. These principles are used by first responders through senior decision-makers, and constitute an all-hazard, scalable, flexible, and adaptable approach to response. The NRF provides the structure to align key roles and responsibilities across the nation, linking all levels of government, non-governmental organizations, and the private sector. The framework provides an overarching coordinating mechanism for accessing federal support for response activities and for specific federal departments and agencies to carry out their responsibilities. Currently, fifteen (15) Emergency Support Functions (ESF) and five (5) Incident Annexes address functional capabilities and resources provided by federal departments and agencies. The NRF is always in effect and elements can be implemented as needed.

While an incident is occurring and after, the priorities are to employ resources to save lives; to protect property and the environment; and to preserve the social, economic, and political structure of the jurisdiction. Depending on the size, scope, and magnitude of an incident, communities, states, and the federal government will be called to action (DHS 2008).

Initial information about an incident will depend on whether the release was overt or covert. An overt release is the intentional release of an agent reported by terrorists, observed by witnesses at the scene of the release, or made known at the time of release by other means. A covert release is the intentional release of an agent not observed at the time the release occurs (DHS 2007a). A biological-related incident may be discovered in one of three ways: 1) discovery of either physical or intelligence evidence (law enforcement actions or suspicious package), 2) detection of an agent through environmental surveillance systems (e.g., DHS BioWatch, US Postal Service Biohazard Detection System), or 3) reports of medical symptoms or disease (Emanuel et al. 2008).

2.2 Phases of an Incident

Effective and timely decision-making in responding to a biological agent incident first requires a broad understanding of all the phases and activities involved. As depicted in Table 1, effective response to a biological release incident comprises numerous elements, grouped into two overarching phases: *Crisis management* and *consequence management*. This mapping is common in response to all chemical, biological, radiological, and nuclear (CBRN) incidents (NSPD 17/HSPD 4, 2002; and DHS 2004). *It is important to note the activities described below do not necessarily occur in sequential order, but may run concurrently, or occur outside the phase in which they are described. Additionally, this document emphasizes the specific activities for the response and recovery to a B. anthracis incident.*

Table 2-1: Phases of an Effective Response

Response and Recovery*					
Crisis Management		**Consequence Management**			
Notification	**First Response**	**Remediation/Cleanup**			**Restoration/ Reoccupancy**
		Characterization	**Decontamination**	**Clearance**	
Receive information on biological incident Identification of suspect release sites Notification of appropriate agencies	Initial threat assessment HAZMAT and emergency actions Forensic investigation Public health actions Screening sampling Determination of agent type, concentration, and viability Risk communication	Characterization of biological agent Characterization of affected site Site containment Continue risk communication Characterization environmental sampling and analysis Initial risk assessment Clearance goals	Decontamination strategy Remediation Action Plan Worker health and safety Site preparation Source reduction Waste disposal Decontamination of sites or items Decontamination verification	Clearance environmental sampling and analysis Clearance decision	Renovation Reoccupation decision Long-term environmental and public health monitoring
* The optimization decision process is applicable to any phase					

Source: Adapted from NSTC (2009)

2.2.1 Crisis Management

The first phase of response and recovery, the *crisis management* phase, involves law enforcement (local, state and federal), first responders (police, fire, and hazardous materials teams), and public health agencies (local, county, state, and federal health) (DHS 2004). The crisis management phase includes measures to identify and characterize the event, as well as to identify, acquire and plan the use of resources needed to respond to the incident. The crisis management phase of the response consists of the initial response activities, which can be further broken down into the *notification* phase and the *first response* phase (DHS 2004).

Depending on the origin of the event, criminal versus naturally-occurring, different agencies will manage the event and different response actions will take place. Law enforcement manages first responses for criminal responses and may designate the incident location as a crime scene while public health manages responses to naturally-occurring events.

At the beginning of the response, the data available depends on whether the release was overt or covert. An observed, overt release is likely to prompt an immediate response including site containment. However, even in those circumstances, the causative agent may not be known. The forensic investigation along with epidemiologic and intelligence data will contribute to the identification of the biological agent. The greatest difference between overt and covert scenarios is an overt scenario more quickly yields greater information about the release (e.g., time, location of the release, dispersion methods) and it leads to a response prior to evidence of exposure or infection in the population. An overt scenario also allows the opportunity to implement public safety measures that may mitigate consequences during the first response phase.

2.2.1.1 Notification Phase

During the *notification* phase, tasks include law enforcement and public health receiving and assessing information, identifying suspected release locations, and communicating key information to the appropriate authorities that, in turn, initiate first response actions (DHS 2006b).

2.2.1.2 First Response Phase

This *first response* phase may involve, particularly in an overt release incident, hazardous material (HAZMAT) and emergency actions, public health response, scene control, law enforcement activities, initial site containment, sampling and analysis, personnel decontamination, and risk communication. HAZMAT and emergency actions are conducted to address any immediate threats to life or valuable property necessary for public welfare (e.g., critical infrastructure), and to establish control of the situation (OSTP 2009). A command post is established, and communication and data exchange between law enforcement and other personnel is performed as needed.

During this phase, data regarding the incident most likely have been generated by numerous responding agencies and organizations, such as HAZMAT teams, law enforcement, including the Federal Bureau of Investigation (FBI) Hazardous Materials Response Team, and public health organizations (state/county/local health departments and CDC). Data from these responding agencies involved in the initial response and investigation will be available to members of the IC/UC and may consist of law enforcement, forensic, and incident reports; preliminary environmental laboratory results; and public health case investigation data.

If results from preliminary samples indicate the likely presence of *B. anthracis* or if law enforcement or public health investigations identify a potential contamination location, the FBI will likely commence a criminal investigation. This criminal investigation may include activities to determine the agent's specific genetic, physical, and chemical properties; search for other types of evidence; establish a possible source of the contamination; and identify the responsible party. If a crime scene is established, the FBI must approve all environmental sampling within the crime scene through the ICS/UC with the ultimate decision for entry into the crime scene made by the Incident Commander (IC). Initial samples are sent to a Laboratory Response Network (LRN) laboratory for confirmatory testing (OSTP 2009). Additional information about LRN laboratories can be found in Section 2.4.5.1.

The DHS National Bioforensics Analysis Center (NBFAC) may also analyze forensic samples (DHS 2006b). Results from the forensic investigation may not be releasable to all federal entities and may not meet the needs of the public health investigation; therefore additional sampling may be necessary during the *first response* phase to obtain information on the presence of an agent and to determine the agent type, concentration, and viability as well as to determine exposure pathways in the building. These activities may continue in more depth during the first phase of *consequence management, which is characterization*.

2.2.2 Consequence Management

The second phase of response and recovery, the *consequence management* phase, is predominantly an emergency management function and includes measures to protect public health and safety, restore essential government services, and provide emergency relief to governments, businesses, and individuals affected (DHS 2004). As the *crisis management* phase transitions into the *consequence management* phase, in which the U.S. Environmental Protection Agency (EPA) plays a critical role and may step in as a lead federal agency, as directed or requested. The main focuses will be on characterization of the contaminated environment, decontamination, and clearance.

The local or state agencies with authority for protecting public health and/or the environment would also likely exert their regulatory authority to assure consequence management efforts are acceptable. *Consequence management* can be further subdivided into *remediation/cleanup*, which includes *characterization, decontamination, clearance*, and *restoration/reoccupancy*. The response and recovery process ends with *restoration/re-occupancy* during which a facility may be renovated, and decisions to allow reoccupation are made by the IC/UC. *However, this phase is not discussed further in this document as environmental sampling does not play a critical role in the restoration process because the building will already have been cleared of contamination.*

2.2.2.1 Characterization

Characterization is the process of obtaining information about a biological agent incident, which is used to determine further action. A sampling plan is developed to characterize the spread of contamination within an area and to obtain semi-quantitative estimates of the biological agent's concentration at specific locations (OSTP 2009). Characterization of an affected site includes describing site-specific characteristics such as, size, construction, heating, ventilation and air conditioning (HVAC) systems, ambient environmental conditions (e.g., temperature and relative humidity), structural materials, stored materials, and contents. If decontamination is warranted, the decontamination strategy decision may be affected by characteristics and materials composition of the specific site as well as the efficacy of decontamination approaches (OSTP 2009).

The information generated from the characterization sampling is used to help modify and refine public health actions developed based on the initial assessment. Uses include estimating the potential exposure to the agent, and deciding where, what, and how to decontaminate (DHS 2006b).

A risk assessment is conducted to determine potential risks posed by the threat agent at a specific site. Risks need to be assessed in order to assist decision-makers in setting clearance goals, planning a decontamination strategy, and developing a sampling plan (OSTP 2009).

Clearance goals will need to be established. Setting clearance goals for a biological agent is not an easy process due to the fact that there are no established reference values (unlike some radiological or chemical agents) or exposure guidelines (OSTP 2009). The IC/UC may choose to assemble a Technical Working Group (TWG), to assist in setting clearance goals appropriate to the site-specific circumstances. The TWG is an advisory group of multi-disciplinary technical experts and scientists that provides input on planning and implementing remediation, including setting clearance goals. The TWG may include representatives from federal, state, local, and tribal agencies, and experts from the private sector or universities. The TWG is an advisory group to the IC/UC, and is not a decision-making body. The TWG provides advice and guidance on such issues as interpretation of analytical results; sampling and analysis plans; selection of the appropriate remediation process and conditions for its implementation; development of procedures for a variety of issues that may arise to address releases and other emergencies during the remediation process; and waste management activities.

2.2.2.2 Remediation

During *remediation*, a decontamination strategy is developed and implemented, taking into account specific information about the agent, incident, and materials to be decontaminated. Ambient conditions (temperature and relative humidity) affect decontamination and must also be considered. After the decontamination approach is selected, a Remediation Action Plan (RAP) is prepared for the site specifying the overall strategy for decontaminating impacted areas and their contents. The decontamination strategy will be a guide for the remediation activities.

Site preparation is necessary before decontamination is carried out. Source reduction can be performed to remove certain items and/or materials from a contaminated site for further treatment and reuse or disposal. Additionally, items and site surfaces may need to be cleaned prior to the main decontamination activity.

After the RAP is complete and approved by IC/UC, the site is prepared and the specific decontamination methods selected for affected site(s) and/or item(s) can be employed. Decontamination is monitored as it is carried out and evaluated as to whether or not the specific parameters were met, goals were achieved, and the operations were conducted successfully.

2.2.2.3 Clearance

The *clearance* phase includes determining whether the agent has or has not been inactivated to the clearance criteria levels. The IC/UC may establish an Environmental Clearance Committee (ECC), which is a group of experts that functions as an independent peer review group. Members of the ECC may be representatives from the local, county and/or state public health agencies, the facility or property owner, local government, and subject matter experts from the EPA, FBI, OSHA, Food and Drug Administration (FDA) and CDC. The ECC conducts a comprehensive review to make recommendations to the IC/UC on whether the clearance goals have been met.

It is important that the ECC be formed as early as possible in the incident so that committee members can become familiar with the situation, review necessary data which may include agent characteristics, extent of contamination, sampling results, decontamination process, and clearance sampling results. The ECC is an independent body that is not part of the decision-making process on decontamination. Clearance sampling and analysis is the ultimate measure of whether decontamination met the criteria outlined in the RAP. If the clearance criteria are met, then decisions will be made on whether to allow unprotected re-entry to a facility and unrestricted use of items in the facility. The IC/UC makes the ultimate clearance recommendation to the lead local public health agency or private facility owner based on judgment as to whether the criteria for decontamination verification and clearance criteria have been met. Ultimately, the facility is returned to the owner/operator.

2.3 Agency Coordination

All levels of government, the private sector, and non-governmental agencies must be prepared to prevent, protect against, respond to, and recover from a wide spectrum of major incidents that exceed the capabilities of any single entity. These hazards require a unified and coordinated national common approach to planning and responding to an incident management. To address this need, Homeland Security Presidential Directive (HSPD) 5: *Management of Domestic Incidents* required the establishment of the NIMS. In addition, Presidential Policy Directive (PPD) 8: *National Preparedness* provides a comprehensive approach to assess national preparedness that uses consistent methodology to measure the operational readiness of national capabilities at the time of assessment, with clear, objective and quantifiable performance measures, against the target capability levels identified in the national preparedness goal.

The NRF specifies what needs to be done to prevent, protect against, respond to, and recover from a major incident. It also specifies how and how well it needs to be done. Together, these related efforts align federal, state, local, tribal, private sector, and non-governmental preparedness, incident management and emergency response plans into an effective and efficient national structure.

2.4 Roles and Responsibilities of Key Agencies, Advisory Groups, and Laboratories

The roles and responsibilities of key agencies, advisory groups, and laboratories are discussed in the following subsections.

2.4.1 Federal Bureau of Investigation

According to the NRF (DHS 2008), the FBI is the lead federal agency for criminal investigation of a terrorism incident. Local law enforcement usually notifies the FBI of a potential Chemical, Biological, Radiological, and Nuclear (CBRN) incident. Other methods of notification can be through local or state public health departments, fire department hazardous material responders, local search warrants where "questionable items" are observed by local law enforcement officers, and the FBI Joint Terrorism Task Force (JTTF) officers.

If initial laboratory results indicate a presumptive positive of *B. anthracis* and/or the circumstances of the incident suggest a credible threat exists, the FBI will commence an investigation including evidence collection. The main objectives for evidence collection are to 1) obtain biological material for further microbiological, chemical, physical and forensic analysis for attribution purposes and 2) locate a dissemination device or other traditional forensic evidence.

If it is a known or suspected biothreat agent incident, the FBI will coordinate with the IC and other entities having jurisdiction (fire department and/or public health department) but will be the lead agency for the criminal investigative response. As part of their investigation, FBI may work with response partners to collect information on the biological agent, including specific genetic, physical, and chemical properties; search for additional items of evidence; establish a possible source of the contamination; and determine the perpetrator(s). For all potential or actual biothreat agent incidents, a Threat Credibility Evaluation teleconference will take place between the local FBI Weapons of Mass Destruction (WMD) Coordinator, the appropriate FBI Headquarters elements (e.g., FBI WMD Directorate, FBI Laboratory, and FBI Critical Incident

Response Group), and other required elements as necessary such as state, locals or other federal agencies.

The determination of whether or not a credible threat exists may not be made until after the initial detection of *B. anthracis*, therefore law enforcement will coordinate sampling efforts with public safety, public health and environmental agencies to preserve the integrity of the material in case it becomes evidence in a criminal investigation (ASTM 2010b). If a crime scene is established, the FBI may form joint task force sampling teams consisting of FBI and non-law enforcement and will approve all sampling plans until the crime scene is released for environmental remediation. This close working relationship is necessary to ensure both the proper collection of evidentiary samples as well as to protect the public health.

2.4.2 State and Local Public Health

State public health programs have primary responsibility for protecting the health and welfare of the public under their jurisdiction. States vary considerably in the nature and scope of the public health services they provide. State governments are responsible for responding to a public health emergency and play certain key roles in preparedness and response. With exception of the largest metropolitan local public health departments, local public health officials will tend to rely on state personnel and capacity for a number of key functions, including providing advanced laboratory capabilities and capacity, and epidemiological expertise, and serving as a conduit for federal assistance. When resources of state and local authorities are overwhelmed, federal assistance can be requested by the affected state.

2.4.3 Department of Health and Human Services (DHHS)/Centers for Disease Control and Prevention (CDC)

Under the NRF (DHS 2008), the Department of Health and Human Services (DHHS), including the CDC and other HHS agencies, has responsibility for public health and medical services. This responsibility provides the mechanism for coordinated federal assistance to supplement state, tribal, and local resources in response to a public health and medical emergency. The CDC engages in all phases of a biological incident. The CDC’s involvement may include:

- Conducting epidemiologic and surveillance activities to identify cases and the populations at risk, and to determine the source of exposure;
- Providing laboratory support for the identification, confirmation, characterization, and drug susceptibility of the biological agent;
- Conducting environmental evaluations to support the epidemiological and surveillance activities and estimate extent of contamination;
- Providing guidance on the identification, diagnosis, and clinical management of human cases;

- Providing guidance on the use of medical countermeasures (e.g., antimicrobials, vaccines, and immunotherapeutics) that may be utilized in response to an event or incident;
- Developing effective infection control practices for communities and healthcare settings;
- Providing guidance on non-pharmaceutical mitigation strategies to assist with the containment and control of infectious agents;
- Providing technical assistance to SLTT, federal and international partners to support public health activities;
- Disseminating key public health and safety messages to the public to provide timely, accurate, clear, consistent, credible, and easily accessible information relevant to the information needs of all stakeholders.

2.4.4 Environmental Protection Agency (EPA)

Under the NRF (DHS 2008), the EPA's actions can include efforts to detect and assess the extent of contamination (including sampling and analysis and environmental monitoring); actions to stabilize the incident and prevent the spread of contamination; analysis of options for the environmental cleanup and waste disposition; implementation of the environmental cleanup; storage, treatment, and disposal of the hazardous materials; implementation of clearance sampling.

As the *crisis management* phase transitions into the *consequence management* phase, EPA may step in as a lead federal agency. The lead agency during *crisis management* may begin to shift the response to EPA, state environmental agencies, cleanup contractors, and consultants working for the facility owners. The main focus will be on characterization and cleanup work.

2.4.5 Integrated Consortium of Laboratory Networks (ICLN)

The Integrated Consortium of Laboratory Networks (ICLN) was established by a Memorandum of Agreement (MOA) signed in 2012 (ICLN 2012). The ICLN is made up of six established laboratory response networks, including the CDC's LRN and EPA's Environmental Response Laboratory Network (ERLN). The purpose of the ICLN is to enable integrated and coordinated response to, and consequence management of, acts of terrorism and other major incidents requiring laboratory response capabilities. A major outcome of the ICLN is the creation of an Integrated Response Architecture that provides, among other things, a framework for incident notifications and updates, preparedness alerts, and situational reports among networks through a secure web portal. Among the roles of the ICLN are to establish methods for risk-based prioritization and to identify and address key gaps in laboratory capabilities. The ICLN also aims to improve capacity for "surge" requirements and efficiencies in laboratory method development and validation.

2.4.5.1 Laboratory Response Network (LRN)

Per the NRF's Biological Incident Annex, biological samples for public health and environmental considerations are analyzed by an LRN laboratory. The CDC LRN comprises approximately 140 labs across the U.S. and several foreign countries. LRN member laboratories and their contact information can be obtained from the LRN program Office, accessible through the CDC Emergency Operations Center.

LRN laboratories are designated as either national, reference, or sentinel. The designation depends on the types of tests a laboratory can perform and how it handles infectious agents to protect workers and the public. The *national laboratories* have unique resources to handle highly infectious agents and the ability to identify specific agent strains. The *reference laboratories* can perform tests to detect and confirm the presence of a threat agent. This allows local authorities to respond quickly to emergencies. The *sentinel laboratories* provide routine diagnostic services and have publicly available microbiology procedures that can be used to rule out suspicion of a biological threat agent in clinical specimens. If unable to rule out the presence of a biological threat agent, sentinel labs are able to safely package and refer specimens to an LRN reference laboratory, thus playing a key role in early suspicion of a covert event. They are not equipped to perform the same tests as reference laboratories.

2.4.5.2 Environmental Response Laboratory Network (ERLN)

EPA's ERLN network (part of the ICLN), consists of federal government, state government, water utilities, and commercial laboratories capable of performing environmental sample analyses for chemical, biological, and radiochemical contaminants to support the EPA's homeland security responsibilities. The ERLN's mission is to provide reliable analytical data for environmental samples of known and documented quality to federal, state, and local decision makers. Such data can then be used to mitigate and recover from releases of toxic industrial chemicals, chemical warfare agents, biological agents, and radiochemical contaminants in environmental matrices collected in support of homeland security incidents. In addition to its own resources, the ERLN leverages other networks' capabilities to support responses related to a biological threat release.

Section III: Sampling Phases during Crisis and Consequence Management

There are four distinct environmental sampling phases during a *B. anthracis* incident: initial response sampling during first response, characterization sampling, verification sampling, and clearance sampling. The purpose and description of each sampling phase are described in this chapter. Sample collection methods used in these sampling phases are described in Section 6 and appendices referenced therein.

3.1 Initial Response Sampling

In situations where release of biothreat agents are suspected, initial response sampling may be conducted by any of a number of entities, such as local HAZMAT or other first response teams, FBI, or public health authorities. The roles these groups may play in initial sampling depend on how the event is uncovered and which group has jurisdictional authority. Most often local HAZMAT or other first response teams are the first on scene. The decision by first responders to collect and submit a sample to the LRN reference laboratory for testing is made at the local level through communication among on-scene responders, the FBI, and the receiving LRN reference laboratory (ASTM 2010b). Their testing typically includes field screening which incorporates field measurements taken early in the site assessment process to identify and delineate the contaminants present (e.g., explosives and radiation), support tactical decision making, and address operational safety measures. Field screening does not include measurements of biological properties. On-site biological assessments to measure properties inherent to biological materials may also be performed in the field using rapid, field-based procedures and assays when a visible powder is present (ASTM 2010b). As a result of the initial risk assessment or first responder testing results, the FBI may determine that there is sufficient indication of a credible threat to assume jurisdiction. The FBI may take immediate tactical actions to contain the threat and mitigate the potential effects until the LRN reference laboratory has received samples and has performed appropriate confirmatory analysis (ASTM 2010b).

The FBI may choose to collect additional samples for forensic purposes; these samples are sent to LRN laboratories or the DHS National Bio Forensics and Analysis Center for definitive analysis (DHS 2006A). The primary objectives of initial response sampling, when conducted by law enforcement personnel, are to identify and confirm if *B. anthracis* is present, and if so, locate the source of the contamination to aid the criminal investigation. Results from the forensic investigation may not be releasable to all federal entities; therefore additional sampling may be necessary. The information from first responders and law enforcement may have important limitations and should be considered with caution (Emanuel et al., 2008), particularly if generated using hand-held assays (see Section 7.1.5 for more information). Also, the forensic investigation is focused on the collection of evidence and the source location and therefore does not involve developing a robust sampling plan. For this reason, a public health sampling plan may be needed to adequately address exposure concerns. If the incident is designated as a crime scene, CDC coordinates with the FBI to ensure appropriate samples are collected to meet public health objectives (DHS 2004).

Initial response sampling for public health purposes focuses on identifying areas of contamination to inform who may have been exposed. Such sampling takes place after confirming *B. anthracis* contamination or when contamination is suspected based on epidemiologic investigation. Information and data from first responders and other groups involved in the initial response and investigation are considered in determining if additional sampling is warranted. In order to conduct an initial assessment of who may have been exposed and identify potential pathways of exposure to support appropriate risk assessment and data-driven recommendations for medical countermeasures, further environmental assessment may be necessary prior to transition to the consequence management phase. Public health sampling actions are independent of the magnitude of the incident, or whether it is overt or covert. Thus, the initial environmental investigation must focus on rapidly evaluating the epidemiological information available (e.g., incident timelines and interviews of those involved). Sampling teams typically utilize a judgmental sampling approach (see Section 5.1) that is intended to maximize the possibility of detecting the presence of any contamination. Comprehensive characterization of potentially contaminated spaces is not a goal of initial response sampling for public health purposes.

Sidebar 1 suggests how initial response sampling might be carried out.

Sidebar 1 – Initial Response Sampling During a Fictional Airport Scenario

A large international airport had a suspicious powder incident associated with a piece of luggage on a baggage carousel giving off a small, steady white cloud of dust. First responders evacuated the baggage claim terminal and cordoned off an area around the baggage carousel. The first responders collected samples that were sent to their local LRN for confirmatory testing. Due to law enforcement intelligence reports, the FBI and local public health responded to the site to conduct additional sampling. Meanwhile, the LRN confirmed a positive sample result for *B. anthracis*. The goal when collecting initial response samples after a confirmed release is to collect samples to evaluate whether contamination is present in other locations and in order to identify who might have been exposed.
It is important to target the location for a plausible pathway that is most likely contaminated. While the immediate area may be perceived to be top priority, other areas and populations should be assessed in order to quickly identify the populations at risk, such as high traffic areas and the area where baggage was offloaded. In addition, *B. anthracis* spores will likely be present on people or baggage in close proximity to the release point and can serve as a fomite to cross-contaminate other areas. For the purposes of this scenario, initial response sampling can determine if the contamination is localized to the baggage claim terminal or if it has spread to other locations (e.g., the taxi stand or food court) (Emanuel et al. 2008). Also, HVAC system return ducts and filters in the immediate vicinity should be sampled in order to assess if contamination spread via aerosol through the HVAC system. Finding surface contamination on the tops of air ducts or rafters that are highly unlikely to have had contact with the contaminated source or finding a dispersion pattern of multiple positive results might suggest that aerosolization occurred during or after the event (CDC2001).

3.2 Characterization Sampling

Characterization sampling is typically used to obtain information concerning the extent and magnitude of contamination to guide remediation. Sampling is used to determine whether an area needs to be decontaminated and what materials need to be decontaminated. The information generated from the characterization sampling is also used to help modify and refine public health actions that were developed based on the initial assessment, if sampling during the characterization phase indicates a different or larger population may have been affected than suggested by initial response sampling.

Characterization systematically expands on the initial assessment findings to identify other contaminated locations and determine the contamination footprint at the affected locations, in order to better define the boundaries. The strategy for the characterization phase is to supplement the information that has already been collected during the initial assessment. The sampling information, specifics of the scenario, and the data collected during the initial assessment may take on many forms and may come from several different groups involved in the initial response. The initial assessment sampling data will be evaluated and reviewed, and information derived from it will be used by IC/UC to assist in formulating the objectives, strategy, and approach for the characterization phase. The information that results from the characterization affects and shapes the planning and implementation of the remediation phase, as determined by the Incident Commander (DHS 2006b).

3.3 Verification Sampling

Overall clearance of an area or building is a multi-step process that includes application of the decontamination technology, verification sampling and other means to follow progress of the decontamination process, and clearance sampling (discussed in following section).

Verification sampling may be performed during the remediation process to establish whether decontamination was effective or sufficient in neutralizing contamination. Verification sampling may include surface sampling using the same methods that are used during the characterization phase. This type of verification sampling would not take place during decontamination but immediately afterward. These samples are collected adjacent to previously identified contaminated surfaces to determine whether the decontamination process has successfully eliminated viable spores where they were previously found.

Sidebar 2 outlines some of the actions that may be taken to monitor the progress of decontamination.

Sidebar 2 – Monitoring the Decontamination Process

In order to monitor the progress and adequacy of decontamination using a fumigant or vaporous decontaminant, biological indicators (BIs) may be used to determine that a particular decontamination reagent has been in contact with specified surfaces or distributed throughout a particular area sufficiently. BIs can be porous cellulose filter pads or stainless steel coupons that have been inoculated with a defined titer of non-hazardous bacterial spores, such as *Bacillus atrophaeus*. *Bacillus* spores, including the *B. atrophaeus* species, are recognized as being highly resistant to inactivation by decontamination processes, including gaseous chlorine dioxide (ClO_2) (Leftman 2008). It is important to note that current BIs are more easily decontaminated than building materials such as carpet. Therefore BIs should not be used alone to determine decontamination efficacy. BIs containing *B. atrophaeus* spores are used in medical applications to demonstrate successful ethylene oxide sterilization, as well as in the pharmaceutical industry to document the effectiveness of small-scale ClO_2 treatment. Following the decontamination process, the BIs will be retrieved and sent for analysis at an independent laboratory to ensure that all spores on the BI were inactivated by the treatment process (Sabre 2007).

Other measurements will be used as well to monitor decontamination operating parameters. For example, during fumigation measurements are collected for temperature, humidity, contact time, and fumigant concentration. These data are used to help determine if the fumigation process has met the criteria established in the RAP and whether it is deemed successful. For surface decontamination technologies such as amended bleach, parameters that may be monitored include contact time, pH, and free chlorine concentration.

3.4 Clearance Sampling

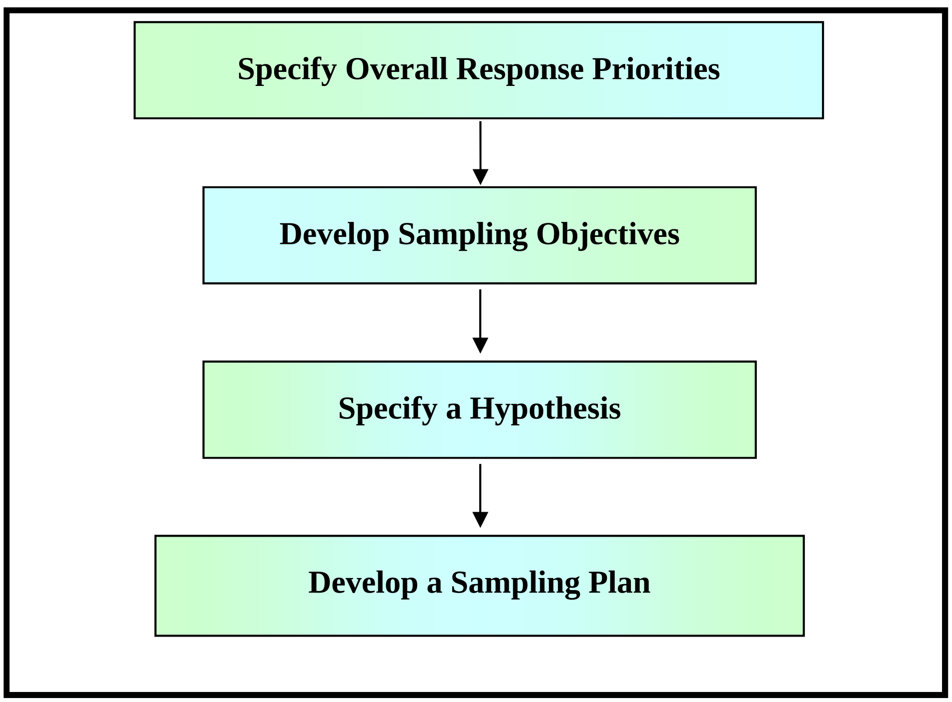
Clearance sampling is environmental sampling that provides a determination of whether clearance goals were met and the facility is ready for final preparations for re-occupancy. In this case, clearance sampling is conducted after decontamination activities are completed but before critical barriers are taken down. In addition, clearance sampling could be conducted in areas where no contamination was found during characterization sampling and thus, no remediation was conducted in those areas. The purpose of clearance sampling is to promote confidence in decision-makers and users of the facility that the facility has been adequately remediated. Consequently, analysis of clearance samples should be done using methods that determine viability of any spores remaining.

After all samples are collected and the sample results are reported, the findings and the methods for verification and clearance sampling used to develop those findings will be presented to the IC/UC. If an ECC was created by the IC/UC, the above information will be provided to the ECC, which will review the findings and then prepare a written clearance statement or document which is provided to the IC/UC. Depending on the impacted facility, the IC/UC or lead local public health agency makes the final decision on whether or not the building is cleared and ready for re-occupancy.

Section IV: Sampling Strategy Roadmap

The first step to produce meaningful sampling data is to understand the basic roadmap for the response (Figure 4-1). The roadmap for the response will include the overall response priorities established by the IC/UC, the sampling objectives, and the sampling approach (Emanuel et al. 2008). The sampling strategy roadmap serves as the framework for developing data of requisite quantity and quality to support an overall process outcome and subsequent decisions.

Figure 4-1. Basic Roadmap for a Sampling Strategy*



*Adapted from Emanuel et al. (2008)

4.1 Specify Overall Response Priorities

Response priorities are set and directed by the IC/UC of an incident. However, the development of these priorities should be made within the context of appropriate information sources. Consideration of critical data streams from law enforcement, intelligence, and epidemiology will help to define the problem, identify what information is lacking, and develop the objectives of environmental sampling. The objectives are met by establishing specific hypotheses and by designing an environmental data collection program that will test the hypotheses.

4.2 Develop Sampling Objectives

Sampling objectives are derived from the response priorities. Establishing clear and tangible sampling objectives that can be translated into sound test hypotheses is critical to determining the amount of data required to draw conclusions. It is also imperative that the data quality requirements are appropriate to support those conclusions. Decisions that are then made as a result of a careful analysis of the data are considered scientifically-based and therefore, provide greater confidence to those making the decisions and to those affected by the incident with regard to their health and safety.

Specific sampling objectives that may be applicable for an environmental data collection response may include (OSHA 2002):

Initial Response Sampling:

- Immediate Assessment of Potential Contamination**:** Determine, in near real-time, whether a release of spores is occurring or has occurred in a facility. Real-time detection instrumentation, biological agents.
- Identifying Spores in a Bulk Material**:** Determine if a bulk material, such as a powder in an envelope, is contaminated with *B. anthracis*. On-site analysis may be used for preliminary assessment, but laboratory analysis provides confirmation.
- Initial Agent Characterization: Determine the identity of the agent, presence of spores, formulation, toxicological properties, antimicrobial sensitivities, strain sub-typing, persistence, and other physical properties.

Initial Response and Characterization Sampling:

- Determining Contamination Pathway: Determine whether spore contamination resulted from airborne or fomite transport.
- Determining Contamination of an Article**:** Determine whether the surface of an article is contaminated.

Characterization Sampling:

- Determining Extent and Location of Contamination (Characterization Sampling): Determine qualitatively, and if possible, semi-quantitatively, the extent and magnitude of contamination; inform the understanding of spore transport and fate; inform decontamination plans, and compare with future clearance sampling results.

Verification Sampling:

- Effectiveness of Decontamination (Verification Sampling): Determine whether parameter measurements for the decontamination technology have met criteria established in the RAP.

Clearance Sampling:

- Post-Decontamination Sampling: Develop a body of data of adequate quantity and quality to enable IC/UC to verify that the originally contaminated environment has been sufficiently decontaminated to allow re-occupancy of the area without the use of personal protective equipment (PPE) or other protective measures (OSHA 2002).

4.3 Specify a Hypothesis

Hypothesis-driven sampling plans provide for defensible decisions based on the resulting data, which can be evaluated semi-quantitatively or qualitatively. Hypotheses should specify the principal question(s) of interest, which will then help identify needed information inputs such as epidemiology data or environmental data. Clear hypotheses must be defined before an investigator decides on the number and types of environmental samples to collect and the specific locations to sample. As data are received and interpreted, this new information may be evaluated against the initial intelligence and epidemiologic data and could refine or shift sampling objectives. Subsequently, a new set of hypotheses may be generated and new sampling plans developed to reflect the changing needs.

4.4 Develop a Sampling Plan

A well-designed sampling plan ensures that resulting data can answer the specific hypothesis being tested, thus fulfilling the sampling objectives. The sampling plan, as defined by the VSPWG (see Section 1.2), is a documented approach for field execution that captures the specific combination of operating precepts and diagnostic tools used for a given scenario to answer a specific hypothesis. A sampling plan is an executable plan of action that addresses the sampling and analytical requirements of a specific situation and is formulated in accordance with the guidance of the sampling strategy. This plan should be developed by experts (e.g., industrial hygienists or environmental scientists with microbial sampling expertise) with training and experience in conducting field studies or investigation.

A sampling plan must specify the sampling approaches, methods, and analyses, as well as the number, types, and locations of samples to be collected in a given physical space. The plan also must address quality control considerations (DHS 2007b). A comprehensive sampling plan cannot be developed prior to an incident because its development is governed by the amount of information known about the agent, whether the location of the release is known, and whether the agent has been modified or enhanced. Only after the sampling objectives are determined and associated sampling approaches (discussed in Section 5) are selected can an incident-specific sampling plan be written. A sampling plan may develop into several individual plans for multiple locations each with a different objective. A sampling plan may be developed for each task or phase of the response (*first response, characterization, remediation,* and *clearance*) (DHS 2007a). Sampling plans should be documented and describe the basis for all steps, including quality assurance.

Sidebar 3 outlines the process EPA uses to document data quality objectives. The format of an incident-specific sampling plan may vary. Appendix F provides an example sampling plan used in the EPA-funded Bioresponse Operational Testing and Evaluation (BOTE II) project.

Sidebar 3 – The EPA Data Quality Objectives Process

In order to ensure that the data collected from the exercise of a sampling plan matches the needs of a hypothesis and related decision, a systematic planning process is needed to design the data collection. As an example, the EPA uses the Data Quality Objectives Process to establish performance or acceptance criteria, which serve as the basis for designing a plan for collecting data of sufficient quality and quantity to support the goals of a study (ASTM 2010c). The DQO Process consists of seven iterative steps that are outlined below and explained in detail within the ASTM document.

Elements of the systematic planning process include:

- Organization: Identification and involvement of the sampling plan manager, sponsoring organization and responsible official, project personnel, stakeholders, scientific experts, etc. (e.g., all customers and suppliers).
- Sampling Plan Goal: Description of the project goal, objectives, and study questions and issues.
- Schedule: Identification of project schedule, resources (including budget), milestones, and any applicable requirements (e.g., regulatory requirements, contractual requirements).
- Data Needs: Identification of the type of data needed and how the data will be used to support the plan's objectives.
- Criteria: Determination of the quantity of data needed and specification of performance criteria for measuring quality.
- Data Collection: Description of how and where the data will be obtained (including existing data) and identification of any constraints on data collection.
- Quality Assurance (QA): Specification of needed QA and Quality Control (QC) activities to assess the quality performance criteria (e.g., QC samples for field and laboratory examinations, audits, technical assessments, performance evaluations, etc.).
- Analysis: Description of how the acquired data will be analyzed (either in the field or the laboratory), evaluated (i.e., QA review/verification/validation), and assessed against its intended use and the quality performance criteria (EPA 2006a).

While these steps are outlined in a sequential fashion, the iterative nature of the DQO Process allows one or more of these steps to be revisited as more information on the problem is obtained (EPA 2002). A good sampling plan should expend no more resources than are necessary to meet the associated objective (EPA 2002).

4.4.1 Sampling Approaches

There are three main sampling approaches described in this document: *judgmental sampling*, *hotspot sampling*, and *combined judgmental and random* (CJR) sampling. Judgmental sampling is environmental sampling in which locations having the greatest likelihood of being contaminated are selected using the investigator's expert judgment (Emanuel 2008). Hotspot sampling is a probabilistic (i.e., samples randomly selected) sampling approach that provides for detecting small hotspots of contamination. The CJR sampling approach employs a Bayesian methodology that combines probabilistic samples with a given number of judgmental samples to obtain a specified level of confidence that a building or area has no detectable contamination.

The type of sampling approach selected is based on the response phase, whether the response is covert or overt, the magnitude and type of release, IC/UC objectives, and the available resources. *Judgmental sampling* is virtually always used in initial public health, characterization and clearance sampling, due to its speed, efficiency, and demonstrated effectiveness. For characterization sampling with the majority of contamination scenarios, judgmental sampling will be sufficient to detect the contamination. However, if *B. anthracis* may have been disseminated in one or more small, isolated locations that would typically not be sampled by judgmental samples, then the *hotspot sampling* approach can be used. Although such scenarios are expected to be rare, the hotspot sampling approach is mentioned as an option for completeness. For clearance sampling, judgmental sampling may be sufficient if clearance statements are made with high confidence based on the effectiveness of the decontamination process along with non-detect judgmental samples. The *CJR approach* can be used for clearance sampling if there is a need to generate statistical confidence statements. A more in-depth discussion of these three approaches is found in Section 5.

In selecting a sampling approach for the site-specific sampling plan, the IC/UC must consider many factors, some of which can be in conflict. The IC/UC must consider the level of confidence in the intended outcome of the process needed (i.e., that the facility is free of detectable contamination), the time available to make that determination, the resources available, and the financial investment required. Components of resources available include staff available to collect samples, consumables used in the sampling and analysis process, and analytical resources (laboratories, staff, and equipment) available for processing of environmental samples. Limitations in any resources required to execute the overall sampling response may limit the options available among sampling approaches that can be exercised in the site-specific sampling plan.

Based on the best available science and the most practical approach, EPA and CDC have developed an *Interim Clearance Strategy for Environments Contaminated with Bacillus anthracis* that considers that the available knowledge of the efficacy of decontamination when combined with a reliance on judgmental sampling alone are sufficient generally to inform positive estimations by the IC/UC on clearance of a previously contaminated facility or space.

4.4.2 Pre-Incident Data

Security camera recordings and eyewitness accounts might provide some information about the extent of contamination. Data on ambient interior conditions (temperature and humidity and time of day) and outdoor conditions (wind speed and direction, temperature, and humidity) contributes to understanding exposure pathways and location of contamination. Such data can be combined with known facility parameters and traffic patterns and used to model the spread of the spores to help estimate exposure of potentially affected individuals. If other environmental sampling systems exist near the site of the incident (i.e., BioWatch collectors), the data from those systems should be obtained and reviewed.

4.4.3 Initial Sampling Plan

Environmental assessment during the initial response phase is a critical component of an overall investigation because it provides important information about the potential exposures to populations who may have been in the release zone. Environmental exposure information along with epidemiological data helps support implementing post-exposure prophylaxis and other public health activities. Knowledge of individuals who have contracted anthrax and whether it is cutaneous or inhalational is important in developing sampling plans. For example, if an individual contracts inhalational anthrax, then that informs the IC/UC that the agent is small enough to enter the deep lung. Thus, the agent may be wide spread throughout the facility. Environmental sampling during the first response phase typically takes place as soon as possible after identifying an incident.

A modular approach provides an effective process for conducting environmental assessment and investigation during the initial response phase as it creates boundaries based on specific questions being asked about the potential incident. Additional benefits include the ability to resolve complex situations, response in the presence of limited resources (on the ground and in the laboratory), and rapid data turnaround resulting from manageable sample numbers. Each module should be designed to address a single hypothesis.

The number and sizes of the modules will depend on the scope and magnitude of exposure. Different modules may exist for:

- Contamination of a closed office environment
- Contaminant drift within an open office environment
- Agent migration via air handling systems

For example, a single individual in an office environment who opens a letter containing a fine powder composed of a *B. anthracis* spores would necessitate a focused investigation of a small area when the interest is exclusively the exposure of one or a few individuals. Environmental sampling would be targeted to provide resolution on the release point and to estimate the level of any potential exposure. However, if people work in an open, cubicle office environment, another module should be established that addresses their exposures resulting from spore drift or contact with contaminated fomites. Consideration of biological agent migration to other areas of the building would be addressed through a separate module with sampling targeted to address cross-contamination as a result of people and fomite movement, as well as air transport through the building's ventilation system (VSPWG 2007; VSPWG 2008; Amidan 2009)). Multiple modules may be employed simultaneously, each addressing unique hypotheses. Some overlap may exist in the sampling strategies for each module. This is acceptable as it assures that the data generated by a given module can be integrated from one module to another to create a seamless picture of an incident. However, too much overlap creates duplication and a waste of limited resources.

This is an iterative process of assessing and responding, and of subsequently modifying the response based on assessment. Certain incidents necessitate an initial response to the crisis before appropriate personnel can complete assessment. Such actions as shutting off air handling systems, restricting access to a location, and initiating post-exposure antimicrobial prophylaxis are time-sensitive and must be started immediately.

While there is no scientific evidence supporting correlation of surface contamination resulting from the deposition of an airborne release of a biological agent and the inhalation challenge that may have occurred to the exposed population during the release incident, it does provide context for identifying which areas likely present the highest risk of exposures to building occupants.

4.4.4 Characterization Sampling: Dividing the Building into Zones

Because resources are limited and remediation must proceed quickly, characterization sampling must be centered on well-defined goals. The most efficient characterization of an incident depends on what is known about the incident. Knowledge and understanding of the spore dispersal mechanism, common transport mechanisms, sampling and analysis resources, and the decontamination techniques available for use will help in developing an efficient characterization sampling plan. For example, if there are small areas with high contamination concentrations that must be identified and addressed using a localized decontamination method, then characterization sampling must be designed to yield a high likelihood of discovering all such hotspots. On the other hand, contamination dispersal could result in a very distributed form, with widespread contamination and few hotspots, as was observed during trials at the Idaho National Laboratory in 2007-2008 involving the aerosol dissemination and sampling of a biological simulant in a building.

The potential for contamination within a large building is often not the same across the entire building. Many factors could affect dispersion patterns including distance from release, air ventilation systems, traffic patterns, building layout, etc. Therefore, the sampling approach could be different in different parts of the building, depending on the likelihood of contamination. In this case, it is helpful to divide a building into zones. A *zone* is defined as an area within a building that has a similar likelihood of contamination, similar building characteristics and the same sampling objectives. The modular approach utilized in the initial response is not based on similar likelihood of contamination, rather it targets areas anticipated to be contaminated and evaluates potential contamination pathways which may or may not be contaminated. These differences in sample results coupled with the location of the sample aid in understanding any potential exposure pathways and persons most likely at risk of exposure.

If remediation is warranted, the IC/UC should be able to choose the type of remediation method(s) to address contamination in the zone. The sampling approach applicable for different zones may be very different for the following reasons:

- The sampling objectives and decision criteria are based on the amount of prior information available for each part of the building, and
- The different zones have different likelihoods of contamination.

Similar zones should have the same sampling approach. Identifying zones and assigning them zone designations should consider any relevant known information regarding the following:

- Building layout
- Ventilation systems and traffic patterns
- Occupant activities
- Release location
- Initial response results and effects
- Apparent contamination pathways
- Within-room features (furniture, counters, tabletop, shelf configurations)
- Surface materials
- Decontamination technology options and areas of influence

Four types of zones are described in the following subsections.

4.4.4.1 Zone 1: Extremely High Likelihood of Being Contaminated
(ASTM 2010d, 2010e, 2010f)

This zone includes areas that are confirmed contaminated or are assumed to be contaminated. Examples include the area around a release point, areas adjacent to the release point with a direct flow path from the release point, and areas in which contamination was detected in the initial response phase.

Because this zone of a building is known or assumed to be contaminated, detailed characterization sampling is not required if the entire zone will be decontaminated. If additional information is needed to support selection of decontamination technologies or parameters in order to decontaminate the entire zone, some additional judgmental samples are recommended. These samples should be located using best professional judgment and should take into account any recommended pre-defined sample locations. If areas of very high levels of contamination require a different decontamination technology than areas of lower levels of contamination, then additional sampling may be required to accurately delineate these areas.

4.4.4.2 Zone 2: High Likelihood of Being Contaminated

By definition, the likelihood of contamination in this zone is high, but there is no obvious evidence of contamination here before characterization. The primary objective for sampling within this zone is to identify contamination if it exists or to provide sufficient confidence that it does not exist. If feasible, in order to quickly determine if the zone is contaminated, first collect some judgmental samples in locations that are most likely to be contaminated. If any of those judgmental samples are identified as contaminated, then this zone can be re-classified as Zone 1 and proceed with the recommendations as discussed in Section 4.4.4.1. If all judgmental samples are uncontaminated, but there is reason to believe contamination may only exist in one or more small hotspots, then the hotspot sampling approach (Section 5.2) could be used if warranted. If the hotspots are sufficiently delineated and are small relative to the entire zone, contaminated boundaries within the zone could be established and decontamination could focus on the contaminated areas within the zone.

If no contamination is found in any of the initial judgmental samples, then a more extensive sampling approach (e.g., judgmental or CJR sampling approaches) may be necessary, if feasible and time permits, so that this area can be cleared. The IC/UC will determine if additional samples must be collected to clear the area. The decision will be based on several factors including, but not limited to, time and resource constraints, feasibility, and magnitude of incident (other buildings potentially contaminated). If all samples show no contamination present, then this zone should have limited or no entry in order to be

protected against potential future cross-contamination.4.4.4.3 Zone 3: Low Likelihood of Being Contaminated

In this zone there is no prior evidence that contamination is present, but there is a low chance of the zone being contaminated. These are areas the IC/UC does not believe are contaminated, but they do not have sufficient evidence to support that conclusion. The characterization sampling objective for this zone is to determine if contamination exists or does not exist. The judgmental sampling approach (see Section 5.1) is recommended.

4.4.4.4 Zone 4: Extremely Low Likelihood of Being Contaminated

This zone includes all remaining areas in the facility that have an extremely low potential of being contaminated because of their location relative to the release point, and the apparent absence of pathways for contamination to travel from the release point. If there is sufficient evidence that there is no known plausible pathway for the contaminant to have entered this zone, then the sampling team is not required to obtain any samples. This zone designation is only included herein for completeness purposes so an entire building or floor plan can be represented, including areas where no samples will be required. If there is some non-negligible chance that contamination is present (albeit a very low chance), then the sampling team should classify the area as a "Zone 3: Low Likelihood of Being Contaminated" and follow the recommended sampling strategy in Section 4.4.4.3.

4.4.5 Clearance Sampling in Designated Zones

The recommended sampling approaches for clearance sampling in Zones 1, 2, and 3 are the same regardless of whether a zone is being cleared after decontamination or after characterization sampling did not detect contamination. The optimum sampling plan for a given incident-specific scenario is a function of various factors, including, at a minimum, the timeframe required for results, the resources available for collecting samples, the resources available to analyze samples, funding available to resolve the situation, and the level of confidence required by the IC/UC and other responsible parties for deciding that a space has been deemed not to be a public health threat.

4.4.5.1 Zone 1: Extremely High Likelihood of Contamination

Zones that were originally classified or reclassified as having an "extremely high likelihood of being contaminated" (Zone 1) require decontamination. After decontamination, such areas within a building are assumed to have a “low likelihood of being contaminated” (Zone 3) and are treated as described in the following section.

4.4.5.2 Zones 2 and 3: High and Low Likelihoods of Contamination

For zones that had a high (Zone 2) and low (Zone 3) likelihood of contamination where all characterization sample results were negative, additional samples may be collected to finally clear the area since decontamination was not conducted in those areas. The IC/UC will determine if additional samples should be collected. The decision will be based on a collection of information including epidemiological data, characterization sampling results, and first responder, law enforcement, and public health information, if time permits. Depending on site and incident specifics, the IC/UC may decide to clear a zone based on non-detect characterization sampling results.

For areas that were classified as Zone 1 and decontaminated, clearance sampling is necessary to demonstrate that there is confidence that no detectable contamination remains, and the area can be released for general use. Because information is known about the locations of contamination identified during the characterization phase, judgmental sampling will always be desirable at or near those locations and surfaces along all potentially contaminated pathways (see Section 5.1). It will be up to the IC/UC to decide whether knowledge, control, and verification, of the decontamination method along with judgmental validation samples will be sufficient for clearance with high confidence.

Regardless of whether contamination was detected and decontaminated, or not detected, another option for clearance sampling is the CJR approach (see Section 5.3). This approach makes use of judgmental and statistical (“random”) samples, and provides for stating with X% confidence that at least Y% of the decision area does not contain detectable contamination. The advantages and disadvantages of the CJR approach are discussed in Section 5.3.

4.4.6 Sensitive Items versus Non-sensitive Items

Some items are considered sensitive due to the fact that these items may be damaged during sampling when using sample collection methods that involve moistening solutions. It is important to determine what items may be considered sensitive by the property owner at the start of the response. Sensitive items can include items such as artwork, photographs, and equipment such as computers, electronic and electrical circuit boards, high-voltage power lines, and electronic control panels. In addition, personal items such as cellular phones, clothing, and jewelry can be considered sensitive items. Many of these items need to be sampled during characterization and removed prior to decontamination of the facility if possible. Sensitive items with a positive characterization sample must be contained before removing so that contamination is not spread. These items can be decontaminated using less destructive methods such as ethylene oxide (only used for small-scale decontamination) at an alternative location. If sensitive items cannot be removed before decontamination, then these items should be protected from the decontaminant. Sensitive items will most likely be sampled using a vacuum sampling technique as was done during the 2001 Amerithrax incident cleanup operations.

4.4.7 Operating Equipment

Decisions must be made on whether equipment (e.g., refrigerators, printers, cash registers, computer screens, typewriters, etc.) present in the contaminated area will be decontaminated and kept after the facility is released for reoccupation, or whether the equipment will be removed and properly disposed. If the equipment is kept, then post-decontamination sampling will be needed to confirm that the equipment has been decontaminated. If there are areas where contamination may have accumulated (such as grease areas or wells, fans, heating or cooling elements, etc.), then samples should be collected in these areas. If there are many small crevices, then vacuum sampling may be in order. If equipment is completely enclosed and air tight, then only wipe sampling of the enclosure will be required.

4.4.8 Optimizing the Sampling Process

At each stage of the response to a contamination incident there are many variables that can be optimized so that only the sampling necessary to achieve the objective(s) is performed. Optimization can be applied in 1) partitioning a facility into designated zones, 2) selecting the sampling approach for each designated zone, and 3) using composite samples where appropriate. The optimization process would be implemented by engaging site workers, technical experts, and key stakeholders to provide the IC/UC with advice on the options, costs and implications of various courses of action.

4.4.8.1 Optimizing via Designated Zones

Sampling can be optimized by partitioning a facility into zone categories, as discussed in Section 4.4.4. Portions of a facility designated as “Zone 1” areas (extremely high likelihood of contamination) may only require minimal judgmental sampling to detect contamination. Spaces designated as “Zone 4” areas (extremely low likelihood of contamination) may require no additional sampling and minimal confirmatory sampling. Areas designated Zone 2 (high likelihood) and Zone 3 (low likelihood of contamination) may receive the most attention during the overall sampling plan development and determination of most appropriate sampling approach for those zones. Consideration will be given to the degree to which these areas are distinct from Zone 1 areas, with an emphasis on passageways between them. Such passageways as open doors or connected air flow systems will increase the amount of sampling done and the approach taken.

4.4.8.2 Optimizing Sample Collection

In many response situations, resources are constrained due to 1) limited laboratory capacity to analyze samples, 2) limited number of people to collect, process, and analyze samples, 3) restrictive cost of the sampling or analysis, and 4) limited sampling media and laboratory processing supplies. Additionally, there may be great pressure to have a quick turnaround on the sample results. When planning sample collection, using the optimization process will ensure that the process is efficient and that the data generated are meaningful and applicable. Iterative-based sampling consists of collecting a set of samples, then using the results to determine where to collect another set of samples. Although this may decrease the number of samples collected, it does require more time then collecting samples for the entire area all at once.

Another optimization process is to collect all the samples at once but then prioritize sample submission to laboratories for analysis. Whether samples are collected all at once from an area or iterative-based sampling is conducted, the IC/UC can prioritize sample submission. Samples that are most likely to answer the sampling hypothesis should be chosen for submission to the laboratory for analysis first. Prioritization of sample submission may be based on:

- Knowledge of the incident
    - Contaminant characteristics
    - High probability sample locations like ventilation filter, electrostatic surfaces, high traffic areas, etc.
    - Epidemiologic data
- Time constraints of the incident
- Overall priority of area/building with respect to response objectives

Composite sampling (discussed below) and pooled sample analyses (see Section 7.3) are two other strategies to reduce the number of samples taken and/or analyses performed

4.4.8.3 Composite Samples

Composite sampling involves collecting samples from multiple locations with the same sample collection device and submitting it as a single sample. This might involve wiping more than one location with the same wipe, or vacuuming more than one location with the same vacuum filter media. The main advantages of a composite sample are the reduction in the number of samples that require processing and analysis and the reduction in the sample collection materials required. Another advantage to composite sampling is the increase of surface area sampled. With discrete sampling the surface area sampled may by 100 cm^2. With composite sampling the surface area sampled may be up to 400 cm^2 which may increase the likelihood of detecting contamination. During the various phases (public health screening, characterization, and clearance sampling) of past *B. anthracis* incidents, composite sampling was used successfully. An example is provided in Sidebar 4, in which composite sampling is used to verify a cross-contamination pathway. With these conditions, collecting composite samples instead of discrete samples (i.e., collecting one sample from one sampling location) should be considered.

Sidebar 4 – Example of Use of Composite Samples

Initial response sampling of a building's entranceways determined that a three-story office building was cross contaminated due to foot traffic from an adjacent contaminated building that had a *B. anthracis* release. The IC/UC decided in advance that all carpets located in large conference rooms would be replaced if found to be contaminated. Therefore, during characterization sampling, the team decided it would save resources by compositing four vacuum sample locations within a conference room as one sample, instead of collecting four discreet vacuum samples. Four locations to serve as one composite sample is given only as a guide and should depend on the area and amount of debris present. Each floor of the three-story building had a large conference room. The sample team collected one composite sample for each conference room on each floor. Composite sampling was deemed acceptable in this scenario because a similar decision would have been made if discrete sampling had occurred. In other words, if only one of four discrete samples was positive the same decision to replace the carpet would have been made.

When sampling multiple locations using composite sampling, the decision makers are treating the one analysis of all those locations as one decision. Collecting multiple composite samples from overlapping sample areas should be avoided. In such a case, the areas cannot be distinguished from each other, all spatial information is lost, and useful information is gained only if both samples are positive or negative.

When collecting composite samples, the following guidelines are provided to maximize the utility of this technique:

- Sample vertical and horizontal surfaces separately.
- Group frequently touched surfaces together, like light switches or door handles.
- Keep similar surface type together (e.g., smooth, non-porous desks and filing cabinets).

If there is a desire to delineate contaminant location by room, then a composite sample should not include locations in two or more rooms. Compositing should only be done within each room. This also holds true for delineating contamination by floor, by ventilation systems, etc. The number of locations to collect with a single sampling media should be between two and six. For swabs only two to four locations is appropriate. This prevents the swab from drying out and minimizes overloading the sampling media, both of which will decrease collection efficiency. For wipes, two or four locations should be collected for the same reasons described above. Composite vacuum samples (e.g., filter sock) should only include two to six locations. More composite locations can be collected with a vacuum sample since the filter sock can collect more material, and it is not subject to drying out since it is not wetted. The number of locations and surface area collected for a given sampling media should be consistent throughout all sampling events. This ensures consistency of results for data interpretation. A composite sample collected from four locations with a single sampling media is referred to as a 4-point composite.

One disadvantage of composite sampling is contamination can be spread from contaminated locations to uncontaminated locations. However, this may not be an issue if finding contamination within an area will lead to decontaminating the whole area. Another potential disadvantage is dilution, which would depend on the sample collection method. Composite sampling using the wipe method may reduce the amount of contamination collected on the wipe from a contaminated location by distributing it to subsequent uncontaminated sampling locations. This may cause a composite sample to be reported as non-detect where otherwise a sample of a single contaminated location would be declared positive. However, composite sampling has been exercised in simulated operational scenarios, such as the second sampling test at Idaho National Laboratory (VSPWG, December 2008), with minimal deleterious effects observed.

The decision to collect discrete or composite samples will be based on the types of decisions made with the results, laboratory throughput, resources (sampling media, sampling personnel) and the size of the incident. In most incidents, collection of both composite and discrete samples will be conducted. Sidebar 5 presents an example of sample collection optimization in order to make a quick decision to fumigate based on refining the sampling strategy to determine if the pathway of contamination was via aerosol deposition. In this example the sampling process prioritizes samples, expedites the timeline, and saves valuable resources for characterizing other zones.

Sidebar 5 – Optimizing Characterization Sampling for Making Decontamination Decisions

A sampling team conducted characterization sampling in an area adjacent to the room where a letter containing *B. anthracis* powder was opened and also shared the same HVAC system. Based on this information, the adjacent area was designated as having an extremely high likelihood of contamination (Zone 1). The IC/UC gathered all existing data from any inhalation and/or cutaneous* cases in that vicinity, in order to develop a sampling plan. Based on analysis of the anthrax cases, epidemiological surveillance, and law enforcement evidence, the team determined no anthrax cases were identified from individuals in this area. There was no evidence that the contaminated letter entered this area. However, the IC/UC hypothesized that contamination could still be present due to foot traffic and possibly by transport via the HVAC system. Sampling this area would help inform those most likely to be at highest risk of exposure, and therefore disease, and help with public health decisions for post exposure prophylaxis (PEP).

The IC/UC decided that if any designated areas had evidence of aerosol deposition, then fumigation would be chosen as the method for decontamination. Therefore, the initial characterization sampling efforts would use the judgmental sampling approach to minimize the time and resources needed to make the decision to fumigate.

The sampling team collected samples on surfaces in the HVAC system that had a high probability for aerosolized spore deposition due to inertial impaction. The surfaces included baffles and downstream (supply air) ductwork where airflow made abrupt changes in direction. In addition, the sampling team collected samples on the downstream (supply air) filters where spore deposition may have occurred. This area of the building also had a drop ceiling that acted as the return air plenum. These return air plenums are good locations for some of the larger spores or agglomerated spores to settle due to slower airflow and longer retention time. Therefore, the sampling team removed ceiling tiles and sampled on top of the tiles as well.

Samples were positive for *B. anthracis* on the supply and return vents and one of the ceiling tiles. Therefore, the IC/UC concluded characterization sampling and initiated fumigation of the area.

* Cutaneous cases would show evidence of surface contamination and inhalation cases would show evidence of aerosolization. Anthrax is not known to be communicable (spread from one infected person to another). (Emanuel et al. 2008).

Section V: Sampling Approaches

Because sampling every surface in a building is not practical, a sampling approach is required to select representative surfaces for sampling. There are three kinds of sampling approaches discussed in this document: judgmental sampling, hotspot sampling, and CJR sampling. Generally, the judgmental sampling approach will be sufficient to detect contamination for first response and characterization sampling. However, the hotspot sampling approach might be needed for characterization sampling to identify smaller, isolated locations of contamination not detected by judgmental sampling. Although the need for hotspot sampling is expected to be rare, it is briefly discussed in this chapter for completeness. The CJR sampling approach employs a Bayesian methodology that combines probabilistic samples with a given number of judgmental samples to obtain a specified level of confidence that a high percentage of a building or area has no detectable contamination. These three sampling approaches are discussed in more detail in the following subsections.

5.1 Judgmental Sampling Approach

Judgmental sampling is environmental sampling in which locations having the greatest likelihood of being contaminated are selected using the investigator's expert judgment (Emanuel 2008). This approach can quickly determine if an area/zone is contaminated, although it is only as good as the information on which sample location selection is based. Using information gathered from the IC/UC, judgmental sampling plans are created with predetermined locations to collect samples. However, sampling teams can also use their judgment to choose new locations while conducting sampling. This approach is commonly utilized during the first response phases involving law enforcement and public health agencies when information to support both the criminal investigation and the implementation of medical countermeasures is needed quickly. Judgmental sampling is also used for the characterization and clearance phases of a response. With judgmental sampling, probability or confidence statements about the absence of contamination are more difficult to make and may require additional assumptions regarding representativeness and likelihood of contamination presence.

Judgmental sampling can be the most efficient way to find contamination if it is either widespread or behaves as expected. Judgmental sampling utilizes expert knowledge on applicable aerosol physics (including particle size, deposition rate, and settling velocity), principles of industrial hygiene, past responses, and epidemiologic and criminal investigations to determine sample locations. It has been successfully used in multiple investigations.

5.1.1 When to Use Judgmental Sampling

Judgmental sampling is often used during the early phases of an incident as the primary sampling strategy. It is most effective to implement during characterization sampling if the source and characteristics of the contaminant are known from the crisis response phase sampling and when supporting epidemiological or forensic data are available. Critical information to consider from the incident, if available, would include the timeline of the incident, the dissemination mechanism, contaminant characteristics, observable contamination, if the HVAC system was shut down and when, any pathways the contamination source moved along, and any critical forensic evidence collected by law enforcement. Even in situations where very little is known about the release, the IC/UC can use professional judgment and draw upon past experience to select sampling locations. Judgmental sampling is also used during clearance sampling.

5.1.2 Selecting Locations for Initial Public Health and Characterization Sampling

As mentioned in Section 4, judgmental sampling focuses on those areas most likely to be contaminated. Different methods of dispersal would result in different patterns of contamination, and sampling should discover the resultant pattern. If the delivery source is known, investigators can quickly identify sampling locations at the source of the release. However, additional sampling locations will need to be identified to determine the extent of contamination in the building or area. If the source is not known, then identifying locations for sampling will be more challenging.

In addition to information gathered from law enforcement, first responders, building occupants, and public health, investigators may inspect the building for visual information to aid in selecting sample locations. Investigators should utilize current knowledge about contamination pathways resulting in spread of the spores through the building to aid in sample location selection. The four primary contamination pathways include:

- Process pathways
- Foot traffic pathways
- Air movement pathways
- Maintenance and other activity pathways

Process pathways are pathways, either manual or mechanical, associated with a work activity or sequence of steps along a given path (Emanuel et al. 2008). For example, the process by which incoming mail in an office building is processed and delivered to individual occupants can provide information on locations to sample. In this scenario, samples should be collected at locations where a contaminated letter or package was known to be present as part of the delivery process (Greene 2002).

Foot traffic movement pathways spread spores from one surface to another or into the air when individuals step in contamination or have contaminated clothing and subsequently move to another location. Samples should be collected along the route individuals took to exit the building (e.g., stairwells, elevators) and the path first responders, law enforcement, and public health took when responding. Paths along which mail carts, equipment, and vehicles moved could also be sampled.

Air movement pathways are pathways associated with the operation of HVAC systems, natural ventilation from open windows, the airflow within affected facilities, and equipment having fans like printers, computers, and refrigerators having a major influence on the spread of the *B. anthracis* spores. The spores can spread quickly throughout the areas served by the same air-handling unit serving the release location, including other floors of the building and all air-handling zones sharing a common return plenum with the release zone. If the HVAC system was operating during the release or was used as the mechanism of dissemination, one can anticipate a greater percentage of the building was contaminated. Specific locations could be sampled including supply air diffusers, return air vent covers, HVAC filters, and equipment fans.

Maintenance and other activity pathways are pathways where actions taken by individuals in these areas can increase or spread contamination in a building. For example, cleaning activities using compressed air or vacuuming can re-aerosolize *B. anthracis* spores. Use of brooms or mops in contaminated areas subsequently used in other areas can cause secondary contamination. In 2006 and 2007, *B. anthracis* contamination was identified in individuals who manually processed imported hides to make drums (Guh 2010, Nguyen 2010). Manually stretching and shaving hairs on contaminated hides resulted in exposure to *B. anthracis* spores.

Sample locations should be selected at sites where *B. anthracis* spores are likely to remain after deposition (repositories). Examples include surfaces with electrostatic charge (e.g., computer screens); tops of light fixtures; tops of signs; air ducts and surfaces near air-supply registers; air return registers, plenums and air-intake grills that are part of equipment cooling systems; ventilation intakes of electronics (e.g., computer tower fans); and HVAC filters.

Knowledge of these four kinds of pathways and likely repositories can assist investigators in identifying sampling locations for judgmental samples.

5.1.3 Selecting Locations for Clearance Sampling

One goal of the sampling approach for clearance purposes is to sample locations where a positive contamination result was found (and/or adjacent locations) in order to verify no detectable spores are present. The rationale is that previously sampled locations having a positive result, and nearby locations, represent the most challenging test of remediation effectiveness. Also, locations more likely to have been previously contaminated (such as surfaces along contamination pathways) can be selected for clearance sampling. This rationale can be applied in cases where a limited characterization was performed because the actual contamination boundary may not have been determined and/or the decision to remediate was made based on positive samples at key locations (See Sidebar 5).

5.2 Hotspot Sampling Approach

The *hotspot sampling* approach uses grid sampling with a random start to provide for detecting a small area of contamination (hotspot). This approach may be needed in characterization situations where a hotspot would not be detected by judgmental sampling. Although such situations are expected to be rare, the hotspot sampling approach is briefly discussed for completeness.

The number of grid samples is chosen to provide sufficiently high confidence (Z%) of detecting a hotspot of a given shape (usually circular or elliptical) and size. The type of grid (square, rectangular, or triangular), the hotspot shape and size, and the confidence parameter are chosen by the IC/UC depending on the specifics of the situation. The VSP software (VSP Development Team 2010, Matzke et al. 2010) implements the calculations for the hotspot sampling approach. For more information about the hotspot sampling approach, see Gilbert (1987, Chapter 10).

5.3 Combined Judgmental and Random (CJR) Sampling Approach

The CJR sampling approach employs a Bayesian methodology allowing investigators to combine probabilistic samples[a] with a given number of judgmental samples to obtain a specified level of confidence (X%) that a high percentage (Y%) of a building, area, or zone has no detectable contamination (Sego et al. 2007, 2010). The Bayesian approach incorporates prior knowledge about the chances of judgmental samples having contamination, so the combination of judgmental and probabilistic samples allows for statistical inferences about the likelihood of there being no detectable contamination. Increased confidence in the conclusion there is no detectable contamination is important in deciding on the need for further public health or decontamination measures following the initial assessment. The CJR sampling approach ensures samples are obtained from the perceived most-likely-to-be-contaminated locations (via judgmental samples) while protecting against the possibility of contamination existing in less likely areas (via probabilistic samples).

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[a] Probabilistic sampling applies sampling theory and involves a randomization aspect in selecting sampling locations.

Probability based sampling applies statistical sampling theory and involves randomized selection of sampling locations. Random sampling locations (or grid samples with a random starting point) can be used to accept or refute statistical hypotheses and to make statistical confidence statements about a decision. However, this approach often requires a large (perhaps impractically large) number of samples to achieve an acceptable level of confidence. The CJR sampling approach, because of using judgmental samples, has the advantage of requiring fewer random samples needed to achieve the same level of confidence. However, this requires making some quantitative statements about the ability of the expert to identify potentially contaminated locations and the likelihood of contamination relative to randomly selected sample locations.

5.3.1 When to Use CJR Sampling

The CJR sampling approach can be used for clearance situations when there is a need to generate statistical confidence statements of the form "There is X% confidence that at least Y% of a decision area does not contain detectable contamination." The clearance situation can be 1) after decontamination of a contaminated area, or 2) without decontamination of an area believed to be uncontaminated. In these cases, the CJR approach selects judgmental samples from locations that are more likely to be contaminated and augments the judgmental samples with probabilistic samples. In the case of clearance after decontamination, locations more likely to be contaminated are those identified as contaminated before decontamination and adjacent locations. In the case of clearance without decontamination, locations more likely to be contaminated include those along contamination pathways based on knowledge of the incident (see Section 5.1).

Sidebars 6 and 7 provide scenarios describing how the CJR approach could be used for clearance sampling after decontamination and without decontamination being judged necessary, respectively. Appendix E provides more information regarding the implementation of combined judgmental and random sampling. *It should be noted that confidence statements as outlined above cannot be directly converted into statements that reflect minimal or no risk to health in the space as there remain no accepted criteria for how clean is safe.*

Sidebar 6 – Clearance Sampling After Decontamination Using the CJR Approach

A small (18,000 square foot) airport terminal was designated as a zone with a "high likelihood of being contaminated." During characterization, judgmental sampling found that one of the plausible pathways (foot traffic) in this zone was contaminated. Judgmental sampling did not detect contamination in other pathways (luggage areas, touch surfaces). The UC made the decision to surface decontaminate the foot traffic areas by first applying amended bleach solution to the entire carpeted floor and then HEPA vacuuming the entire carpeted floor surface. The UC decided that if decontamination was successful, the carpet would remain in place for reuse. All other fixtures and surfaces in the airport terminal remained and were not remediated.

Now that cleanup was performed, the UC wants to state that they are 95% confident that at least 99% of all surfaces do not have detectable contamination. During clearance sampling, the judgmental sampling accounted for 5% of the carpet being vacuumed and samples were collected in those locations most likely to be contaminated (dense foot traffic areas). The carpet vacuum samples consisted of vacuuming 100 locations each three foot by three foot. All 100 judgmental samples were reported as non-detect for *B. anthracis.* The sample planners believed that any judgment sample on the floor was twice as likely to identify contamination than any uninformed random sample. It was determined that judgmental samples were twice as likely to identify contamination because the characterization sample identified that contamination was most likely spread via foot traffic. Based on the total surface area and the likelihood that judgment samples are twice as likely to identify contamination, the total number of samples collected included 100 judgment and 762 random samples. If all sample results are negative, then the "95% confident that at least 99% of all surfaces do not have detectable contamination" can be stated.

Sidebar 7 – Clearance Sampling Without Decontamination Using the CJR Approach

A small (1,800 square foot) building was entirely designated as a Zone 3 with a "low likelihood of being contaminated." During characterization, five judgmental samples (three foot by three foot vacuum samples) were reported non-detect for contamination. As a result, no remediation was performed. After characterization sampling the building was protected from becoming contaminated by other nearby operations. This was done in order to use the characterization judgmental samples as part of the clearance sample numbers. The UC wants to state that they are 95% confident that at least 95% of the floor surface does not have detectable contamination.

The sample planners decided not to assume the building was clean and assigned the expected *a priori* probability that all areas have no detectable contamination as 50% (unknown). They believed that any judgment sample collected was twice as likely to be contaminated as any uninformed random sample. The CJR approach resulted in judgmental sampling on 5% of the carpeted floor surface (for a total of ten, three foot by three-foot vacuum samples) and an additional 27 random vacuum floor samples. Combining the five judgmental samples from characterization, a total of 37 vacuum samples were analyzed. If all sample results are negative, then the "95% confident that at least 95% of all surfaces do not have detectable contamination" can be stated. If there were any positive results, then decontamination strategies should be implemented to remediate the area.

Section VI: Sample Collection

The sampling objectives described in Section 4.2 contribute to deciding on the sample collection method(s) selected. A variety of sampling devices are available and one should be selected based on the location and type of surface to be sampled. The selection of appropriate environmental sample collection methods that can meet the sampling objectives must include consideration of the following factors (OSHA 2002):

- Laboratory capability and capacity to process expected samples
- Recovery efficiency of the sample processing method, specificity and sensitivity of the analytical method, and a determination of the need for quantitative, semi-quantitative, or qualitative results
- Suitability of the sample collection method for the potentially contaminated surface
- Cost effectiveness and efficiency of the sampling plan in meeting stated objectives

Obtaining as much information as possible about the *B. anthracis* spores to be sampled, their physical characteristics, and how they were released will help ensure that the most appropriate sample collection method(s) is employed.

Because the methods for sample processing depend on the analytical laboratory, the LRN laboratory to be used (discussed in detail in Section 7.1) must be contacted during the initial planning stages of sampling to discuss method selection. The final decision to select specific sampling methods, media, and materials should be made in conjunction with the LRN.

6.1 Sample Types

This section describes the following sample types: bulk material, surface, air, liquid, and soil samples. The specific uses and advantages of each sample collection method are also described in this section.

6.1.1 Bulk Samples

Bulk sampling is used to collect a visible solid material to determine the presence of a biological agent including *B. anthracis*. Bulk sampling can be used during any phase of an incident (EPA 2006a). Bulk samples of the source contaminant could be used to determine the characteristics of *B. anthracis* spores (ASTM 2010a).

Bulk samples can be collected in a variety of ways, but must be coordinated with the receiving laboratory. Loose source material (i.e., powder) can be collected by placing material into a sterile vial using a sample spoon, trowel, or spatula. Alternatively, sections of carpet or upholstery can be removed and transported to the laboratory for processing and testing (Anderson RL, 1982).Portions of HVAC filter media, or clothing that may be contaminated with *B. anthracis* may also be collected and sent to the lab. A method for collecting bulk samples is described in Appendix C.

6.1.2 Surface Samples

Surface sampling involves collecting microbial contaminants from a surface using an appropriate sampling device to determine the presence of *B. anthracis* spores. Swabs, wipes and vacuum filter socks or cassettes are the primary collection devices for spores on surfaces and are used during all phases (identification, characterization, decontamination, and clearance) of a response (CDC 2012a).

Determining the most appropriate type of surface sample collection method depends on whether porous or non-porous surfaces are to be sampled. Wipes and swabs should be used on non-porous surfaces while vacuum socks or filter cassettes should be used on porous surfaces (DHS 2006A). Examples of non-porous surfaces include: stainless steel, painted wallboard, glass, floor tile, and wood laminate. Examples of porous surfaces include: ceiling tile, fabrics, carpet, clothing, rugs, and upholstered furniture.

When collecting samples for *B. anthracis* on porous surfaces, use of wipes can be considered, because some studies have demonstrated higher recovery efficiencies (RE) when wipes were used to sample carpet and upholstery than when vacuum methods were used (Buttner et al. 2004, Estill et al. 2009, Valentine et al. 2008). Rayon/polyester or cellulose/polyester blends are superior to cotton wipes (Valentine et al. 2008). Vacuum sampling is also effective for spore collection from carpet or upholstery and could be used on these surfaces if high concentrations ($> 10^2$ spores/cm^2) are expected (Brown et al. 2007a).

Certain solutions (wetting agents) can be used to pre-moisten biological collection devices to enhance their overall performance. Common solutions include sterile water, sterile saline, neutralizing buffer, sterile phosphate buffer, and peptone buffer. In addition, surfactants (such as Tween 80, Tween 20, or pleuronic) can be added to these pre-moistening solutions to improve removal of spores from surfaces. Neutralizing solutions block the continued action of a disinfectant after sampling. These neutralizing solutions are important during post-decontamination activities (verification and clearance sampling) to ensure that samples, when analyzed properly, are not falsely negative due to the presence of residual disinfectant. Among available neutralizing solutions are:

- Butterfield’s buffer with 0.02% Tween 80 (Tween 80 is effective in neutralizing phenolic compounds and acting as a surfactant);

- Dey Engley broth (Becton Dickinson, Sparks, MD) [neutralizes chlorine compounds and iodine, but may encourage growth during transport];

- Neutralizing Buffer (Becton Dickinson) [contains sodium thiosulfate to neutralize chlorine compounds and aryl sulfonate complex to neutralize quaternary ammonium compounds];

- Letheen broth (Becton Dickinson [neutralizes quaternary ammonium compounds, but may encourage growth during transport]; and

- Phosphate Buffered Saline, pH 7.2 with 0.02% Tween 80 [Tween 80 is effective at neutralizing phenolic compounds and acts as a surfactant].

Similar recovery efficiencies (26.8 ─ 39.0%) have been obtained with wipes pre-moistened with each of these neutralizing buffers that were processed by the LRN laboratory processing procedure (see Appendix B). The choice of neutralizing solution depends on the disinfectant used. During the initial identification and characterization of a contaminated building, collection devices with a neutralizing solution are less important.

There are factors that will affect the choice of which wetting solutions to use for pre-moistening swabs and wipes for sampling. For example, phosphate-containing solutions (e.g., Butterfield's buffer and phosphate buffered saline [PBS]) may inhibit polymerase chain reaction (PCR) assays if appropriate DNA extraction and purification is not performed; sterile water could lyse osmotically sensitive vegetative cells; and the use of Dey Engley or Letheen broth may encourage growth during transport. PCR techniques are discussed later in the document in Section 7.1. Sterile saline will not neutralize the action of a sporicide or chemical. However, if it is used during characterization sampling (on surfaces that do not contain sporicides), it may help to preserve the viability of *B. anthracis* spores.

Some of the sampling devices can be purchased pre-moistened or they can be pre-moistened prior to collecting a sample. CDC recommends the use of a neutralizing buffer as the pre-moistening solution in their validated swab and wipe-sampling and analysis methods (CDC 2012a). The CDC developed methods for processing macrofoam swabs and cellulose sponge wipes from samples collected on environmental surfaces. These processing protocols use traditional culture methods and yield semi-quantitative estimates of the amount of *B. anthracis* contamination on a sample The CDC collection procedures for the validated swab and wipe method and a non-validated gauze method are provided on the CDC website at www.cdc.gov/niosh/topics/emres/surface-sampling-bacillus-anthracis.html.

6.1.2.1 Swab Samples

Swabs are appropriate for sampling small [< 100 square centimeters (cm^2)] non-porous surfaces. Swabs work best for small areas like crevices, corners, supply air diffusers, air return grills, and hard-to-reach places. The CDC currently recommends using synthetic or macrofoam swabs for the collection of *B. anthracis* spores on smooth, non-porous surfaces (CDC 2012a). The LRN laboratories are capable of processing samples collected in accordance with this sample collection protocol using the prescribed swab type.

6.1.2.2 Wipe Samples

Wipes are appropriate for sampling larger (> 100 cm^2) non-porous surfaces, such as walls, desks, and non-carpeted floors. Wipe sampling can be performed using either cellulose sponges or gauze. The CDC currently recommends using a cellulose sponge wipe for the collection of *B. anthracis* spores on smooth, non-porous surfaces (CDC 2012a). The LRN laboratory or laboratories that will be analyzing the sponge wipe samples should be consulted prior to using this collection method to determine if that laboratory is capable of processing and analyzing the sample.

6.1.2.3 Vacuum Samples

The primary sample collection method for sampling large porous surfaces (> 600 cm^2) for *B. anthracis* spores is vacuum sampling. Collecting samples by vacuuming is advantageous for covering large, non-porous surfaces and porous surfaces such as carpeting, ceiling tiles, ventilation systems filters, and upholstered furniture. This type of sampling also works well for capturing bulk powder or dust in hard-to-reach places. Vacuum sampling is also the best choice for sensitive items such as electronics and personal items, since it is less likely to cause damage compared to pre-moistened swabs and wipes. The LRN laboratory or laboratories analyzing the vacuum sampling devices should be consulted prior to using this collection method to determine if that laboratory is capable of processing and analyzing the sample. Currently, vacuum sampling and analysis methods have yet to be validated.

During vacuum sampling, bulk material is trapped by the dry collection media/filter by utilizing a small, HEPA vacuum cleaner or a small sampling pump to draw air through the filter. A number of sampling devices can be used to collect samples from porous materials including filter socks, 3M Forensics Vacuum filters, or 35 mm cassettes. The filter sock method utilizes a filter sock and attachment nozzle that fits onto the inlet nozzle of a HEPA vacuum hose. The 3M Forensics Vacuum filter is favored by law enforcement groups due to its ease of use in evidence collection protocols. This filter also attaches to a HEPA vacuum cleaner hose for sampling, though care should be exercised to regulate the power of the vacuum so the filter integrity is not compromised during sampling. The last option uses micro-vacuuming techniques to collect a sample using personal sampling pumps or carbon vane pumps. These pumps utilize a suitable filter contained in a closed-face, conductive sampling cassette to which a short section of plastic tubing cut at a 45° angle is added to the inlet. The EPA method for collecting vacuum sock samples is described in Appendix C. Information on proper packaging and shipping of vacuum socks can be found on the CDC website (CDC 2012b).

Vacuum samples must be collected using only HEPA vacuum cleaners. Conventional home or industrial vacuum cleaners should not be used for sample collection because they can further disperse spores if filtration is insufficient.

6.1.3 Air Samples

Air samples can be taken to determine 1) the extent of airborne contamination, 2) whether *B. anthracis* spores have migrated from the contamination zone, and 3) whether *B. anthracis* spores are still detected in the air after remediation. The primary methods for collecting airborne *B. anthracis* spores include filter media, impactors, and liquid and dry impingers. Sampling using filter media is the type of air sampling most commonly used, whereas the impinger method of sampling is rarely used. The need for data on viable versus non-viable spores should be evaluated prior to selecting an air sampling method (DHS 2006b). Commercially available air samplers and methods for collecting air samples are summarized in Appendix C.

6.1.3.1 Aggressive Air Sampling

AAS is a methodology used to confirm a negative finding of contamination in a space either as part of a public health investigation or as part of the clearance phase process after decontamination of a known contaminated area. The method involves 1) vigorous agitation of the surfaces in a space (using leaf blowers, for example) to aerosolize any particles, and 2) high-volume air samplers to acquire and concentrate aerosolized materials for analysis. The method also uses oscillating fans to keep any *B. anthracis* spores suspended. AAS originated as a testing method for asbestos abatement jobs. AAS is usually only performed after all the surface sampling results have been analyzed and results are negative. However, it is performed before removing critical barriers and negative-air units. As previously mentioned, in some situations, surface sampling may not be conducted for clearance, and AAS may be the only method used. AAS can be an important tool to determine the potential of *B. anthracis* spores to become re-entrained into the air from surfaces following the application of an energy source. Since inhalation is the exposure route of most concern, AAS was used as a final step in demonstrating the effectiveness of the remediation process in many of the 2001 anthrax terrorism incident bio-remediation projects (McKenna and Intrepido 2008). Used correctly, it provides an additional level of testing and complements the surface sampling to provide an overall more rigorous test and may add to the preponderance of evidence that a facility is free of detectable contamination.

6.1.4 Liquids and Soil

If applicable, soil and liquid samples can be collected using a variety of methods and equipment to assess whether they are contaminated with spores of *B. anthracis*. The sampling objective is to determine if any soil or liquids (e.g., decorative fountains, potted plants, and plumbing fixtures) are contaminated. This type of sampling can also be used as a tool for initial confirmation of contamination and evidence collection. Various methods for collecting liquid samples are described in Appendix C. When collecting soil, confer with the analytical lab for appropriate methods.

6.2 Sampling Team

Sampling teams should be composed of personnel who are trained to work with hazardous materials in a hot zone (a zone that contains, or is suspected to contain, highly virulent infectious organisms) (NFPA 2008, CFR 1994). The use of experienced investigators to conduct environmental sampling will provide the greatest likelihood of locating and identifying *B. anthracis* spores, if present. Additional information on sample data documentation and data management is found in Appendix D. Personnel should be trained in the appropriate disciplines necessary for sample collection, including sampling methods, equipment, and materials; knowledge of building systems; dissemination pathways; aerosol-generating procedures/equipment; and decontamination methods. As described in Section 6.2.1, a Health and Safety Plan (HASP) should be established at the site. Personnel should also be trained on the use of PPE, safety precautions, and hazards associated with sampling, and included in a medical program.

Personnel and team configurations may vary and should be optimized based on incident-specific requirements. A minimum of two persons is essential for conducting sample collection using aseptic techniques to minimize cross-contamination of the sample and any potential evidence.

6.2.1 Safety and Health

Individuals collecting environmental samples place themselves at substantial risk of exposure. Sample collection personnel work within suspected contaminated environments and their sampling activities may mobilize and even cause re-aerosolization of the *B. anthracis* spores. Therefore, precautions to protect investigators should be implemented prior to conducting an environmental sampling response. A HASP should be developed that includes the following elements: medical monitoring, training, and appropriate selection and use of PPE. Elements of a comprehensive medical program include medical countermeasures, medical screening, monitoring, and follow-up care. These recommendations can be found in a number of separate guidance documents that are referenced below. These documents should be reviewed prior to developing and implementing a HASP.

Relevant safety and health guidance documents are:

1. *Use of Anthrax Vaccine in the United States: Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009* (CDC, 2010) (http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5906a1.htm?s_cid=rr5906a1_e)

2. *Protecting Investigators Performing Environmental Sampling for Bacillus anthracis: Personal Protective Equipment* (NIOSH website accessed May 2012) (http://emergency.cdc.gov/agent/anthrax/environment/investigatorppe.asp)

3. *Recommendations for the Selection and Use of Respirators and Protective Clothing for Protection Against Biological Agents* (NIOSH, 2009) (http://www.cdc.gov/niosh/docs/2009-132/default.html)

4. Stern EJ, Uhde KB, Shadomy SV, Messonnier N. Conference report on public health and clinical guidelines for anthrax [conference summary]. Emerg Infect Dis [serial on the Internet]. 2008 Apr. (http://www.cdc.gov/EID/content/14/4/07-0969.htm)

5. *OSHA Anthrax E-Tools* (OSHA website accessed May 2012) (http://www.osha.gov/SLTC/etools/anthrax/index.html)

6.2.2 Aseptic Techniques

Aseptic technique is the operation or performance of a procedure or method under carefully controlled conditions to reduce the risk of exposure and prevent the introduction of unwanted material/matter (contamination) into a sample (ASTM 2010b). Aseptic sampling requires the designation of collector (sampler) and an assistant (assistant sampler or facilitator) who coordinate sample collection, packaging, and documentation. The assistant sampler is responsible for providing the sampler with the appropriate tools and facilitating collection. For example, opening and handing materials to the sampler as required, including sample collection containers, gloves, sampling media, other sampling materials, and packaging materials, as well as performing any administrative functions including communication, photography (FBI Laboratory Publication, Handbook of Forensic Services 2003), as well as ensuring the sample collection sheet is filled out. The sampler should be the only person to come in contact with the suspect *B. anthracis*. The sampler is also responsible for signing the final chain-of custody form outside of the hot zone.

A critical element of aseptic sampling is the sampler and assistant sampler must have a new pair of non-powdered, nitrile or vinyl examination gloves for each sample collected. This layer of gloves is in addition to the gloves are part of standard PPE ensemble (that is, team members will have three or more layers of gloves on) for each sample collected. During sample collection involving direct contact with the collection media (e.g., gauze wipe), it is recommended for responders to wear sterile gloves to avoid introduction of any other organisms to the sample. The use of sterile gloves is not recommended when using sample collection devices not requiring direct contact with the collection media (e.g., swab or sponge with handle). Regardless of the sampling device selected, the gloves must be changed between samples.

6.3 Sample Collection Quality Assurance/Quality Control

Field blanks and media blanks (also referred to as negative controls) are taken for data authentication (EPA 2002) and should be submitted to the laboratory with other samples. Field blanks are used to identify and estimate sample contamination, which may occur immediately before and after sampling (evaluation of protocols), during shipment, or while awaiting measurement in the laboratory. Field blanks should be collected during sampling to enable determination of any cross-contamination that may occur due to techniques used by the members of the sampling team. It is good practice to collect one field blank for every 10 samples collected. Media blanks are unexposed samples, not taken to the field or shipped, used for background correction of sample readings or for recovery studies. Media blanks should also be submitted with samples for analysis ensuring the sample media had not been contaminated prior to sample collection. A discussion with the laboratory regarding the number of media blanks to include with the samples should also take place. Approximately 1 to 5 media blank samples should be included for each media type or lot number. Media blanks ensure each lot of medium is sterile and free of contamination. Field sampling teams should have standard operating procedures requiring the collection of field and media blanks. The CDC sample collection procedures (CDC 2012a) describe the collection of field and media blanks.

The following quality assurance procedures also apply:

- All instrumentation must be operated in accordance with operating instructions as supplied by the manufacturer unless otherwise specified.
- Equipment checkout/calibration activities occur prior to sampling/operation and must be documented.
- All mechanical equipment should undergo routine maintenance according to manufacturers' specifications.
- A regular schedule for maintenance and equipment upkeep should be coordinated for each piece of equipment.

- Sampling equipment should be verified to be in working order prior to deploying with the environment sampling teams
- Potential cross-contamination should be minimized between samples.

6.4 Chain of Custody

A Chain of Custody (CoC) form documents transfer of sample custody from one individual to another, from the time the sample is collected until final analytical disposition. Each individual in possession of the sample must be noted by recording their signature on the form. The CoC record should include instructions for the laboratory technician as to analytical methods, potential dangers, and any pertinent handling procedures that should be observed. The CoC form should be kept separate from the sample (i.e., should not be placed with the sample) in order to preserve appropriate CoC. The CoC record must include at least the following information:

- All available information regarding the potential hazards associated with the agent;
- Handling procedures associated with the samples;
- Sample identification number;
- Sample concentration, if known;
- Sampling location;
- Collection date and time;
- Sample matrix;
- Names and signatures of the samplers; and
- Signatures of all individuals who had custody of the samples (EPA 2006a).

6.5 Information on Sample Packaging and Shipping

Environmental samples collected for the purpose of determining whether *B. anthracis* spores are present should be considered "Infectious Substances." As such, the shipper is responsible for establishing protocols to ensure these samples are correctly identified, classified, packaged, labeled, marked, documented, and shipped according to applicable federal and international regulations (ASTM 2010a). These regulations include:

- Public Health Service, 42 CFR Part 72,
- Department of Transportation,
- 49 CFR Parts 171-178,
- United States Postal Service, 39 CFR Part 111, and
- International Civil Aviation Organization (published by the International Air Transport

Association), Dangerous Goods Regulation.

Investigators who handle and transport infectious substances must receive training on the preceding regulations prior to collecting samples for submission to an LRN laboratory. Triple-layer packaging (consisting of a watertight primary container, watertight secondary packaging, and a durable outer packaging) may be required.

Section VII: Analytical Considerations

Consulting with the laboratory prior to selecting sampling and analysis methods is essential. When establishing sampling guidelines, an open dialog must be held with the laboratory to determine what requirements and procedures they may have. The samplers should discuss with the laboratory the number of samples expected to be collected, the sample collection methods, and the estimated time of sample delivery (EPA 2006a).

The analytical laboratory should provide information about:

- Names, contact information, directions and any special laboratory-specific instructions (forms, etc.)
- Guidance on preferred media and buffer solutions and discussion on the types and quantity of sample to be collected
- Chain of Custody (CoC) forms and requirements to deliver and drop off samples at the laboratory (EPA 2006a, ASTM 2010a)

The limiting factor in sample collection and analysis is the capacity of the laboratory to process and analyze these samples. Although the sampling team may be able to collect a large number of samples during a single sampling incident, the number of samples collected should be balanced against laboratory capacity and should be spread over a sufficient number of LRN laboratories to ensure adequate turnaround time to results. If it is not feasible to spread sample collection (and hence, sample analyses) out over a few days or to refer samples to a larger number of LRN laboratories, it will be necessary to prioritize samples so they may be processed and analyzed in a staged manner to achieve the result of sequential sampling (Emanuel et al. 2008).

7.1 Analytical Methods

A variety of methods are available for processing and analyzing samples for *B. anthracis*. The method for analysis of samples is selected based on the phase of the incident (purpose of sampling) and the time frame the results are needed. In some cases, multiple methods may be utilized to analyze a sample. It is up to the sampling plan coordinator in conjunction with the laboratory to determine the most appropriate method to suit the needs of the incident. In a bioterrorist attack, detection of *B. anthracis* is performed in a step-by-step manner. An overall response usually involves:

1) Presumptive and rapid analysis of limited, judgmental samples in the hot zone using on-site biological assessments such as hand held assays;
2) Confirmatory analysis of samples in the contaminated area using microbiological culture, biochemical, serological, and PCR to identify *B. anthracis*;

3) Characterization of the extent of contamination using analytical methods such as PCR, immunoassays, and/or where feasible, culture followed by PCR or immunoassay.
4) Post-decontamination analysis of samples to determine presence or absence of viable (live) *B. anthracis* using a combination of microbiological culture, PCR, Rapid Viability PCR, and immunoassay methods

7.1.1 Standard Microbiological Laboratory Culture Method

Microbiological culture is a method of growing a microorganism for identification and determining concentration in the sample being tested. Culture on solid medium employs Petri dishes containing an agar-based growth medium for the growth of bacteria. Bacteria will grow as colonies on the surface of the medium. In the case of *B. anthracis,* each individual colony represents the growth of a single spore or a clump of spores. Another method is broth culture, in which spores are inoculated into a liquid nutrient medium. The microbe must be viable in order to grow on either solid or liquid medium. These methods usually take days before any confirmatory answer is available.

Culture is the gold standard for determining the presence of viable *B. anthracis*. Therefore, it is used during the initial response phase to confirm the presence of viable spores and during clearance sampling to confirm no viable spores remain after decontamination. Theoretically, culture can detect the presence of a single viable spore within a sample. Thus, culture has a lower limit of detection (LOD) than either an immunoassay (e.g., hand held assay) device or a nucleic acid amplification method (e.g., PCR).

7.1.2 Real-Time PCR-Based Analytical Methods

PCR is a method used for detecting *B. anthracis* DNA, which can provide presumptive results from a direct sample in 3 to 6 hours but does not assess spore viability. DNA amplification methods such as PCR depend on the hybridization of primers to their complementary sequences in the target gene of the test species. Once hybridized, DNA polymerase (e.g., Taq DNA polymerase) amplifies the target sequence millions of times in an hour, so the target gene is detected if present. Although PCR is both sensitive and specific, it is susceptible to inhibition by various compounds found in environmental matrices. This inhibition can result in a false negative result by contaminating DNA from the target organism (Buttner et al. 2004). PCR requires the laboratory to have specific equipment and the necessary supplies (e.g., primers and probes) to conduct the *B. anthracis* analysis. Neither the CDC nor the FBI recommends testing samples in the field using commercially available field PCR methods for the detection of *B. anthracis* spores (OSTP 2002).

Due to limitations associated with inhibition to compounds in the environment and the inability to identify viable spores, PCR analysis is not utilized to determine when clearance goals have been met. PCR is primarily utilized during the initial response phase while awaiting culture results which requires additional time for results and during characterization sampling. Characterization sampling is conducted after viable spores are identified during initial public health/law enforcement sampling identifies viable spores. In this case, PCR samples are assumed viable.

7.1.3 Comparison of Culture and Polymerase Chain Reaction Methods

Culture analysis is the principal method for determining quantitative information and is considered to be the definitive method for identifying the presence of viable spores. Culture analysis generally requires days to obtain data when compared to PCR, where results can be obtained within hours. The culture method is less expensive but also requires additional laboratory equipment, such as vacuum manifold systems to perform filter plate testing.

There are numerous advantages of PCR-based methods over traditional culture methods, including: 1) rapid detection, 2) specificity ─ critical selection of target genes and design of primers and probe provide detection at a single species level, 3) detection of agents in complex environmental samples in collection buffer, 4) detection of difficult-to-grow agents, 5) analysis of inactivated agents ─ samples suspected of containing highly potent and contagious agents can be inactivated before analysis by PCR, and 6) multiple gene targets per agent and multiple agents can be detected by multiplex PCR assays, thereby allowing high-throughput sample analysis. However, PCR-based analytical methods cannot determine the viability of *B. anthracis*. Additionally, the number (concentration) of spores or cells present in a sample by PCR cannot be determined. Comparison of features of culture versus PCR methods are presented in Table 7-1.

Table 7-1. Comparison of Culture versus PCR for *B. anthracis*

Culture	PCR
Theoretical sensitivity of one spore	Lower detection limit is 50 - 100 spores
Requires organisms to be viable	Organisms can be viable or non-viable
Growth media has shelf life of 30 - 60 days	Primers and probes for real-time PCR available in LRN laboratories and have a shelf-life of 2 years (dehydrated and stored at 5°C) and 6 months (rehydrated and stored at 4°C)
Results available in 32-40 hours (includes time to subculture for purity)	Results available in 3 to 6 hours but delays may occur depending on the number of samples that are run
Results are considered by CDC to be definitive after PCR confirmation	Results are considered by CDC to be presumptive on direct sample but are confirmatory on pure culture
Growth of contaminating micro-organisms can mask target	Fewer problems with a large number of micro-organisms
Less expensive when compared to PCR	Susceptible to inhibition by compounds found in environmental matrices
No additional laboratory infrastructure required	Additional laboratory infrastructure required (e.g., separate rooms for extraction and amplification)

7.1.4 Rapid Viability PCR (RV-PCR)

The Rapid Viability PCR (RV-PCR) method is most useful for the analysis of samples collected during and after cleanup/decontamination because determining the presence or absence of viable (live) *B. anthracis* spores (in the presence of large number of inactive spores) is a key analytical requirement during this phase of response (Létant 2010, 2011). The method involves extraction of spores from sampling medium, permitting them to germinate in a culture broth, and using real-time PCR to detect the growth of viable bacteria. The RV-PCR method integrates high-throughput sample processing, short-incubation broth culture, and highly sensitive and specific real-time PCR assays to detect low levels of viable *B. anthracis* spores in the presence of challenges including high levels of dead spores, high levels of live, non-target cells and spores, and high levels of dust. This method can be more sensitive than the traditional culture-based method because the whole processed sample is used for analysis. It is relatively rapid, cost-effective, less labor-intensive, less prone to inhibition by environmental matrices, and less prone to interference by outgrowth of other bacteria, fungi, other microbes, and other biological material in the sample. It also provides higher-throughput and generates significantly less bio-hazard and other laboratory wastes than the culture-based method.

7.1.5 Hand-Held Assay-Based Immunoassay (HHA)

HHAs, also known as Test Tickets or Smart (Sensitive Membrane Antigen Rapid Test) Tickets, are hand-held devices containing small chromatographic strips. The device exposes the strip to possible contamination, and then indicates whether contamination was detected. They are also known as Immunochromatographic Lateral Flow Assays. They are the most user-friendly assays and mostly used for preliminary screening of samples in the field. Usually, these tests take approximately 15 minutes. However, the detection specificity has been inferior to other detection methods and has led to false positive results. The Executive Branch does not recommend field-testing using commercially available HHAs for the detection of *B. anthracis* spores (OSTP 2002). Results from such on-site biological assessments are not public health actionable, meaning decisions regarding public health action are pending until confirmatory testing is completed. The DHS's Science and Technology Directorate continues to work to improve the specificity and sensitivity of these commercial field test kits and HHAs, and this strategy will be updated as new information becomes available.

7.2 Method Validation

Method validation is the process of proving a sampling method or analytical method is acceptable for its intended purpose (EPA 2002). The International Organization for Standardization (ISO) defines validation as the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled. Validation includes the characterization of the method performance parameters including accuracy, precision, ruggedness, robustness, sensitivity, specificity, LOD, limit of quantification, reproducibility, linearity, and range (EPA 2006a). Using validated methods is important because it minimizes inconsistencies in the collection, transport, extraction, and analysis of samples. It enables a better interpretation of results and lends itself to comparison of results between independent incidents.

At present, two methods for sampling and analyzing *B. anthracis* on non-porous surfaces have been validated (Hodges 2010, Hodges 2006, Rose 2004, Rose 2010). These methods utilize a cellulose sampling sponge and a macrofoam swab as the sampling media. The collection protocols are available to the public on the CDC website (CDC 2012a).The laboratory processing protocols have been shared with all of the LRN laboratories via a secure website and these laboratories are trained and equipped to analyze these samples.

7.3 Optimizing Sample Processing and Analysis

Sample analysis can be optimized depending on the type of information sought from the sample analysis. If qualitative data are acceptable during the initial characterization phase and it is presumed that any *B. anthracis* spores are viable, each sample can be quickly processed and analyzed (within hours) for *B. anthracis* signatures using PCR analytical techniques that provide information on the presence of the DNA of the agent in the sample. Performing such an analysis may require running a second, more specific analysis in order to determine viability by culturing the sample, which generally requires an additional 16-20 hours to obtain results for *B*. anthracis. The IC/UC will determine whether all or a portion of the samples will be analyzed by PCR and/or culture. PCR analysis is regarded as a qualitative analysis method in that results provide the presence or absences of DNA signatures in the sample. Quantitative culture analysis provides an estimate of the magnitude of contamination of viable spores. The magnitude of contamination is important for selection of the decontamination method and evaluating the efficacy of the decontamination technology by comparing pre- and post-decontamination sample results. PCR analysis during characterization sampling can expedite the sample analysis and save the laboratories valuable time and resources.

Another optimization process that can be conducted is batching or pooling sample analyses. The pooling or batching of sample analyses is performed by combining a number of similar discrete samples in the laboratory after individually collected samples have been prepared for analysis. An aliquot of each sample’s elution is combined and analyzed as one sample. The main advantage of pooling samples for analysis is the reduction in the number of analyses that must be performed; however, certain laboratory processing steps still occur on each individual sample. The principal disadvantage is that combining the eluent from many samples essentially dilutes the portion that will be cultured from each sample which in turn raises the amount that is needed to be present in positive samples to ensure detection, hence, increases the risk of a false negative result. As a rule of thumb, site characterization sampling (i.e., prior to determining whether to decontaminate a space or not) presents the most beneficial case for pooling samples, since contamination has a higher likelihood of being present. If the result is positive and details about the specific location of the positive sample are needed, individual analysis can be done on each individual sample. Pooling of samples may not be beneficial in the post-decontamination phase, since spore concentrations should be lower after decontamination and the risk of a false negative result due to dilution is substantially increased

While pooling samples for analysis can make best use of available analytical resources, care should be taken when deciding which samples to pool for analysis. It should be done by the IC/UC in a logical manner (similar to composite sampling) that is consistent with the level of delineation desired between areas, surfaces or locations. Additionally, current validated analytical procedures do not consider pooling of samples. Advice and recommendations on the details of procedures for preparing and analyzing pooled samples should be secured from technical experts and/or the validating authority to ensure that the process used and the results are rational and comprehensible extensions of validated methods. The decision to pool samples for analysis will be based on the type of information that can be gained from the results.

7.4 Sample Transportation and Storage

Because samples must be transported to the laboratory, and processing and analyzing samples takes time, test results will not be immediately available. Samples should be transported to the laboratory as quickly as possible. Results are reported within hours or days after samples are submitted depending on the type of analysis conducted.

The *B. anthracis* viability and stability when collected with moist samples (swabs, wipes/sponges) depend on the wetting agent. Endospores are not likely to germinate in saline- or buffer-moistened collection media. However, the sample matrix may influence germination if sufficient nutrients are present. In general, samples should be transported to the laboratory at 5^{o} C and analyzed as rapidly as possible to minimize the loss of viability and maintain sample integrity. Samples should be refrigerated upon arrival to the laboratory at 5°C until the sample can be processed. The lower temperature also minimizes endospore germination (DHS 2007b). CDC conducted a shipping integrity study on macrofoam swabs (see Appendices B and C). Based on the findings of the study, CDC recommends sampling media (cellulose sponges and macrofoam swabs) are shipped on ice or on cold packs in order to maintain a temperature between 2° ─ 8°C (Perry 2010). CDC recommends processing samples within 48 hours of sampling to ensure maximum recovery of spores (Rose 2010).

Dry specimens for determining the presence of *B. anthracis* spores may consist of “bulk” powders or vacuum samples. These specimens should be stable as long as they are kept dry, in the dark (to avoid ultraviolet exposure) and shipped at ambient temperature. Once in the laboratory, they can be stored in a cool, dark, dry place until analyzed. The length of time they can be stored without loss of viability may depend upon the sample matrix and the presence of sporicidal agents (DHS 2007b).

7.5 Laboratory Analysis Quality Assurance/Quality Control

Generation of analytical data of known and documented quality is a critical factor in the accurate assessment of and appropriate response to *B. anthracis* contamination incidents. Generating data of sufficient quality requires analytical laboratories to: 1) have trained personnel, 2) acquire and maintain required supplies, equipment, and reagents, 3) conduct the appropriate quality assurance QA/QC procedures to ensuring all measurement systems are in control and operating, 4) document all analytical results, and 5) document analytical QA/QC procedures and corrective actions.

In general, analytical QA/QC requirements for pathogen methods include an initial demonstration of measurement system capability, as well as the capability of the laboratory and the analyst to perform the method with the required precision and accuracy. Ongoing analysis of control samples should also be performed to ensure the continued accuracy and reproducibility of the analytical results. QA/QC procedures should be performed each time a test is performed to ensure the quality of analytical results.

7.6 Interpretation of Data

Sample preparation methods have varying extraction efficiencies. This means some methods are better at extracting the *B. anthracis* spores or *B. anthracis* DNA from the sample matrix than other methods. Thus, the efficiency of the sample preparation method should be reviewed with the laboratory so that there is an understanding of the extraction efficiency. This information is crucial when combined with knowledge of the LODs of the analytical method (EURACHEM 1998). A LOD is the smallest amount of analyte that can be distinguished from background with 95% confidence. In addition to extraction efficiency, various sample collection methods have various recovery efficiencies. Recovery and extraction efficiencies may depend on the concentration of contaminant, the type of surface to be sampled, and the sample collection method. Hence, *B. anthracis* could be present in the environment and yet not detectable by the analytical method because of low recovery and/or extraction efficiencies, as well as analytical uncertainties. Ideally, the LODs of the analytical method for the range of samples it will be applied to should be low enough to detect with high confidence levels of a biological agent at or above risk-based exposure limits (DHS 2006b). Currently, there are no data to support a risk-based exposure limit for *B. anthracis* (Hong, T., P. L. Gurian, and N. F. Dudley Ward, 2010.).

After the laboratory has completed analysis of the samples, they must perform appropriate validation testing of their results and evaluate them for data surety and authentication prior to submission (Emanuel et al. 2008).

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Appendix A

CDC Surface Sampling Procedures for *Bacillus anthracis* Spores from Smooth, Non-Porous Surfaces

Surface sampling procedures for *Bacillus anthracis* spores from smooth, non-porous surfaces

May13, 2010
Revised April 26, 2012

GENERAL

These sampling procedures were prepared by the Centers for Disease Control and Prevention (CDC) to provide standardized methods for industrial hygienists, or other trained samplers under the direction of sampling experts, to use when sampling for *Bacillus anthracis* (*B. anthracis*) spores. These procedures supersede previous CDC procedures for collecting environmental samples for *B. anthracis*, including *Comprehensive Procedures for Collecting Environmental Samples for Culturing Bacillus anthracis*, which were developed during the 2001 anthrax terrorist events. As stated in that guidance, CDC planned to update the guidance as new information becomes available. In particular, one major change is the recent efforts by CDC to validate methods for the laboratory processing and analysis of *B. anthracis* spores.

These procedures are meant to be used for collection of samples on smooth, non-porous surfaces and can be used in both indoor and outdoor environments. Examples of non-porous surfaces are stainless steel, painted wall board, floor tile, or wood laminate. Each sampling method has its specific uses and advantages. Sampling methods must be coordinated with the laboratory to ensure that they are ready to accept and process all the samples. This is particularly important if deviating from the validated sampling procedures.

Swabs are appropriate for sampling small surfaces or hard to reach locations of less than 4 inches square (in^2), like crevices, corners, supply air diffusers, air return grills, and hard-to-reach places. Wipes are appropriate for sampling larger non-porous surfaces including walls, desks, and floors. An interagency effort known as the Validated Sampling Plan Working Group (VSPWG) is currently developing a document titled *Reference Guide for Developing and Executing Bacillus anthracis Sampling Plans in Indoor Settings*, which outlines approaches and methodologies to characterize and guide remediation of indoor sites with potential or actual contamination. The VSPWG document, once published, should be consulted for additional information about strategies and guidance for sampling *B. anthracis*. This document will also assist with the interpretation of results for samples collected with macrofoam swabs and cellulose sponges on smooth, non-porous surfaces following these collection procedures.

Note: Additional information regarding *Bacillus anthracis* sampling, including recommendations for protecting investigators while sampling, are available at emergency.cdc.gov/agent/anthrax/environment/ and www.cdc.gov/niosh/docs/2009-132/default.html.

The collection of samples associated with a crime scene or suspicion of a criminal event should be coordinated with law enforcement authorities. Sampling teams need to be aware that samples collected may be or become criminal evidence, and certain additional procedures associated with sample collection will be necessary.

MACROFOAM SWAB PROCEDURE

SWAB MATERIALS

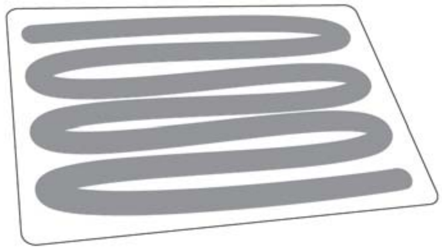
1. Gloves, nitrile
2. Ruler, disposable, and masking tape or sample template, disposable, sample area size 4 in^2 (26 cm^2)
3. Macrofoam swab, sterile, 3/16 inch thick medical-grade polyurethane foam head, 100 pores per inch, thermally bonded to a polypropylene stick (such as the Sterile Foam Tipped Applicators Scored with Thumb Stop [Puritan, Guilford, Maine; catalog number 25-1607 1PF SC] or equivalent)
4. General neutralizing buffer that will inactivate halogen disinfectants and quaternary ammonium compounds, 10 milliliter (mL), sterile (such as the Neutralizing Buffer [Hardy Diagnostics, Santa Maria, California; catalog number K105] or equivalent)
5. Screw-cap centrifuge tubes, sterile, 15 mL (such as 15 mL High-Clarity Polypropylene Conical Centrifuge Tube [Becton Dickinson, Franklin Lakes, New Jersey; catalog number 352097] or equivalent)
6. Sample labels or permanent marker
7. Re-sealable plastic bag, 1-quart or smaller
8. Re-sealable plastic bag, 1-gallon or larger

SWAB SAMPLING PROCEDURE

1. Wearing a clean pair of gloves over existing gloves, place the disposable template over the area to be sampled and secure it. If the template cannot be used, measure the sampling area with a disposable ruler, and delineate the area to be sampled with masking tape.
2. Remove the sterile swab from its package. Grasp the swab near the top of the handle. Do not handle below the thumb stop.
3. If the sterile swab is not pre-moistened, moisten the sterile swab by dipping it in the 10 mL container of neutralizing buffer solution. Remove any excess liquid by pressing the swab head on the inside surface of the neutralizing buffer solution container.

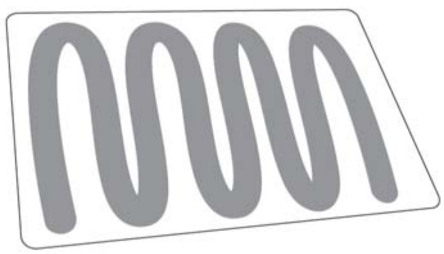
    Note: Once a sterile swab has been moistened, the remaining neutralizing buffer solution and container must be discarded.

4. Swab the surface to be sampled using the moistened sterile swab. Use an overlapping 'S' pattern to cover the entire surface with horizontal strokes.

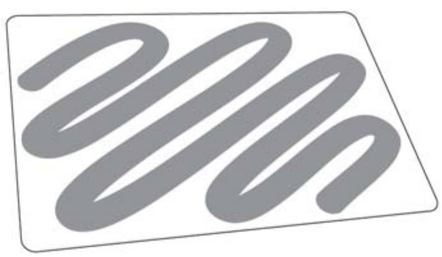


Note: Depending on the design of the swab, a rolling motion can be used when swabbing the surface to maximize swab contact with the surface.

5. Rotate the swab and swab the same area again using vertical 'S'-strokes.



6. Rotate the swab once more and swab the same area using diagonal 'S'-strokes.



7. Place the head of the swab directly into a sterile screw-capped centrifuge tube. Break off the head of the swab by bending the handle. The end of the swab handle, touched by the collector, should not touch the inside of the tube. Securely tighten the screw-cap and label the tube (e.g., unique sample identifier, sample location, initials of collectors and date and time sample was collected). Collection tubes and re-sealable bags may be pre-labeled to assist with sampling efficiency.
8. Place the sample container in a re-sealable 1-quart plastic bag. Securely seal and label the bag (e.g., sample location, date and time sample was collected, and name of individual collecting the sample).

Note: Remove excessive air from the re-sealable plastic bags to increase the number of samples that can be shipped in one container.

9. Dispose of the template, if used.
10. Remove outer gloves and discard. Clean gloves must be worn for each new sample.

CELLULOSE SPONGE PROCEDURE

CELLULOSE SPONGE MATERIALS

1. Gloves, nitrile
2. Ruler, disposable, and masking tape or sample template, disposable, sample area size 100 in^2 (645 cm^2)
3. Sponge, sterile, pre-moistened with 10 mL neutralizing buffer solution, 1.5 by 3 inches cellulose sponge folded over a handle (such as the 3M™ Sponge-Stick [3M, St. Paul, Minnesota; catalog number SSL-10NB] or equivalent)[a] or sponge, sterile, dry, 1.5 by 3 inches cellulose sponge folded over a handle (such as the 3M™ Sponge-Stick [3M, St. Paul, Minnesota; catalog number SSL-100] or equivalent) and general neutralizing buffer that will inactivate halogen disinfectants and quaternary ammonium compounds, sterile, 10 mL (such as the Neutralizing Buffer [Hardy Diagnostics, Santa Maria, California; catalog number K105] or equivalent)
4. Screw-cap specimen container, sterile, individually wrapped 4 ounce (such as General Purpose Specimen Container [Kendall Healthcare, Mansfield, Massachusetts; catalog number 8889-207026] or equivalent)
5. Sample labels or permanent marker
6. Re-sealable plastic bag, 1-quart or smaller
7. Re-sealable plastic bag, 1-gallon or larger

CELLULOSE SPONGE SAMPLING PROCEDURE

1. Wearing a clean pair of gloves over existing gloves, place the disposable template over the area to be sampled and secure it. If a template cannot be used, measure the sampling area with a disposable ruler, and delineate the area to be sampled with masking tape. The surface area sampled should be less than or equal to 100 in^2 (645 cm^2).
2. Remove the sterile sponge from its package. Grasp the sponge near the top of the handle. Do not handle below the thumb stop.
3. If the sterile sponge is not pre-moistened, moisten the sponge by pouring the 10 mL container of neutralizing buffer solution over the dry sponge.

   Note: The moistened sponge should not be dripping neutralizing buffer solution.
   Note: Any unused neutralizing buffer solution **must** be discarded.

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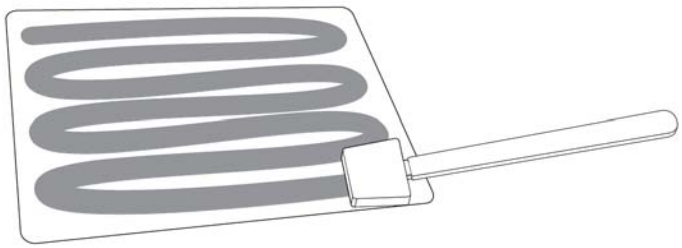
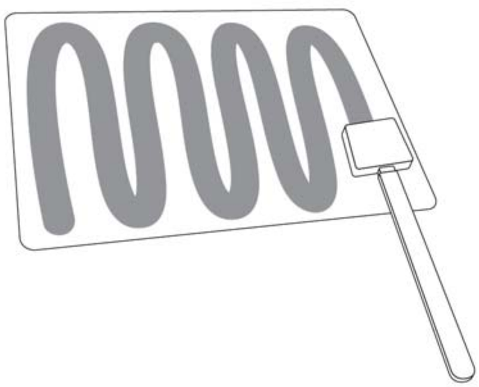
[a] Additional sponges with limited recovery efficiency data available include the Versalon Non-Woven All-Purpose Gauze Sponge (Kendall Healthcare, Mansfield, Massachusetts; catalog number 8042), Bacti-Sponge (Hardy Diagnostics, Santa Maria, California; catalog number SK711), Cellulose Sponge with DE Broth (Solar Biological, Ogdensburg, New York; catalog number BS-10BPB-1), and Sponge-Wipe (Micronova, Torrance, California; catalog number SWU-99 [cut into 2 by 2 inches).

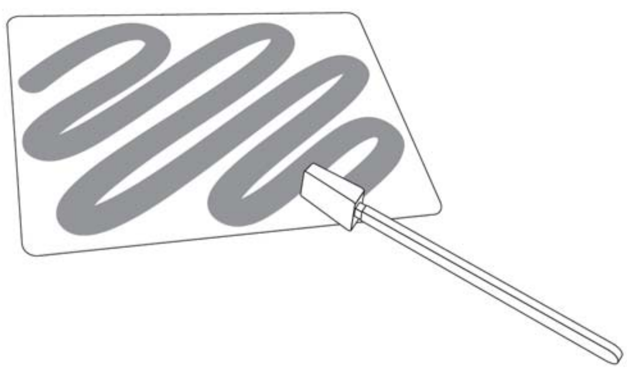
4. Wipe the surface to be sampled using the moistened sterile sponge by laying the widest part of the sponge on the surface, leaving the leading edge slightly lifted. Apply gentle but firm pressure and use an overlapping ‘S’ pattern to cover the entire surface with horizontal strokes.



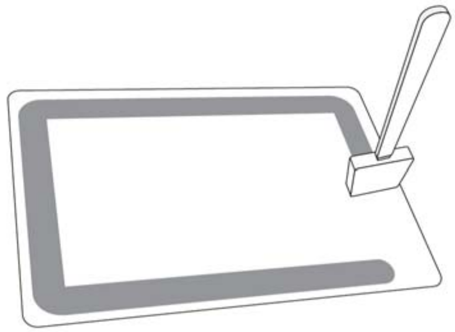
5. Turn the sponge over and wipe the same area again using vertical ‘S’-strokes.



6. Use the edges of the sponge (narrow sides) to wipe the same area using diagonal ‘S’-strokes.



7. Use the tip of the sponge to wipe the perimeter of the sampling area.



8. Place the head of the sponge directly into a sterile specimen container. Break off the head of the sponge by bending the handle. The end of the sponge handle, touched by the collector, should not touch the inside of the specimen container. Securely seal and label the container (e.g., unique sample identifier, sample location, initials of collector and date and time sample was collected).
9. Place the sample container in a re-sealable 1-quart plastic bag. Securely seal and label the bag (e.g., sample location, date and time sample was collected, and name of individual collecting the sample). Specimen containers and re-sealable bags may be pre-labeled to assist with sampling efficiency.

   Note: Remove excessive air from the re-sealable plastic bags to increase the number of samples that can be shipped in one container.

10. Dispose of the template, if used.
11. Remove outer gloves and discard. Clean gloves should be worn for each new sample.

GAUZE PROCEDURE

GAUZE MATERIALS

Note: This sampling and analytical method has not been validated by CDC. A standard sampling procedure is provided in the event that the macrofoam swab or cellulose sponge methods cannot be utilized.

1. Gloves, nitrile
2. Gloves, sterile, nitrile
3. Ruler, disposable, and masking tape or sample template, disposable, sample area between 144 in^2 (929 cm^2)
4. Gauze, sterile, non-cotton, polyester blend sponge or rayon/polyester blend, 2 by 2 inches (such as the Versalon All-Purpose Sponge [Kendall Healthcare, Mansfield, Massachusetts; catalog number 8042; includes two gauze squares/packet] or equivalent)
5. General neutralizing buffer that will inactivate halogen disinfectants and quaternary ammonium compounds solution, 10 mL, sterile (such as the Neutralizing Buffer [Hardy Diagnostics, Santa Maria, California; catalog number K105] or equivalent)

6. Pipette, 5 mL, sterile, individually wrapped (such as the Greenwood Products' Sterile 5mL Standard Transfer Pipette [Greenwood Products, Inc., Middlesex, New Jersey; catalog number GS137038] or equivalent)
7. Screw-cap specimen container, 4 ounce, sterile, individually wrapped (such as General Purpose Specimen Container [Kendall Healthcare, Mansfield, Massachusetts; catalog number 8889-207026] or equivalent)
8. Sample labels or permanent marker
9. Re-sealable plastic bag, 1-quart or smaller
10. Re-sealable plastic bag, 1-gallon or larger

GAUZE SAMPLING PROCEDURE

1. Wearing a pair of gloves over existing gloves, place the disposable template over the area to be sampled and secure it. If the template cannot be used, measure the sampling area (144 in^2) with a disposable ruler, and delineate the area to be sampled with masking tape.
2. Partially peel open the sterile gauze package carefully exposing the gauze.

   Note: The sterile gauze should not be touched without sterile gloves.

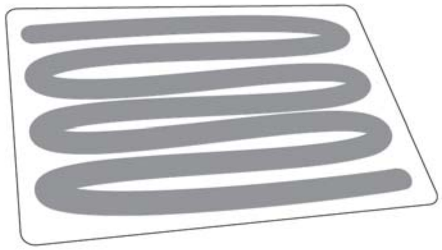
3. Measure 5 mL of neutralizing buffer solution from the 10 mL container using a disposable pipette and apply to sterile gauze in its original packaging. Remove outer gloves.
   Note: The moistened gauze should not be dripping neutralizing buffer solution.
   Note: Any unused neutralizing buffer solution and the pipette **must** be discarded.

4. Don a pair of sterile gloves.

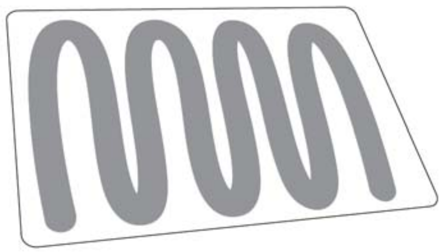
   Note: Sterile gloves are required when sampling with gauze because of the direct contact with the sampling media.

5. Remove one of the sterile gauze (if two per package) and dispose of or retain the other gauze as a field blank (see section 4.1).
6. Completely unfold the remaining moistened sterile gauze, and then fold in half.
7. Wipe the surface to be sampled using the moistened sterile gauze, fingertips should be held together and apply gentle but firm pressure. Use an overlapping 'S' pattern to cover

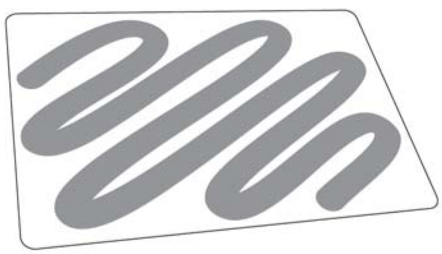
the entire surface with horizontal strokes.



8. Fold the exposed side of the gauze in and wipe the same area again using vertical 'S'-strokes.



9. Fold the exposed side of the gauze in once more and wipe the same area using diagonal 'S'-strokes.



10. Fold the gauze, exposed side in, and place it into a sterile screw-cap specimen container.
11. Securely tighten the screw-cap and label the container (e.g., unique sample identifier, sample location, initials of the collectors and date and time sample was collected).
12. Place the sample container into a re-sealable 1-quart plastic bag. Securely seal and label the bag (e.g., sample location, date and time sample was collected, and name of individual collecting the sample). Specimen containers and re-sealable bags may be pre-labeled to assist with sampling efficiency.

Note: Remove excessive air from the re-sealable plastic bags to increase the number of samples that can be shipped in one container.

12. Dispose of the template, if used.
13. Remove outer gloves and discard. Clean sterile gloves should be worn for each new sample.

BLANKS

FIELD BLANKS

Field blanks are samples handled exactly the same as those used to collect field samples, except they are not allowed to come into contact with the contaminated surface. It is used to estimate contamination arising from preparation for sampling, sampler technique, and shipment and storage prior to analysis. The number of field blanks collected should be equal to at least 10% of the samples collected. Field blanks should be collected while in the contaminated area. While wearing clean (sterile gloves for handling gauze), the macrofoam swab, cellulose sponge, or gauze should be removed from its packaging, moistened (as needed, see sections 3.1, 3.2 and 3.3), and then placed in the appropriate container (either a centrifuge tube or specimen container). An aliquot of the unused portion of the opened neutralizing buffer solution should also be collected when using macrofoam swab, cellulose sponge, or gauze media that are not pre-moistened.

MEDIA BLANKS

Media blanks are unexposed samples used for background correction of sample readings or for recovery studies. Provide two unopened sample media (macrofoam swab, cellulose sponge, or gauze) per lot used and provide two unopened, unused samples of the neutralizing buffer solution (if not using pre-moistened media) as media blanks to the processing laboratory.

DECONTAMINATION

SAMPLE BAG DECONTAMINATION

1. Place multiples of the re-sealable 1-quart plastic bags into a 1-gallon re-sealable plastic bag. Securely seal the 1-gallon re-sealable plastic bag and label the bag (e.g., identify samples contained in the re-sealable plastic bag, sample locations, date and time samples were collected, and name of individual collecting the samples).
2. Decontaminate the outer surface of the larger re-sealable plastic bag using a fresh pH-adjusted bleach solution (household bleach diluted 1:9; pH-adjusted to 6.8-8.0) with a 10-minute contact time before the re-sealable plastic bag leaves the contaminated area. This solution can be prepared by:

   Step 1: Mixing one part household bleach (5.25 to 6.0 % sodium hypochlorite) with 5 parts water (v/v);

   Step 2: Adding 1 part white vinegar; and

   Step 3: Adding 3 parts of additional water.

   Note: Additional information about decontamination is available at www.epa.gov/opp00001/factsheets/chemicals/bleachfactsheet.htm.

3. Thoroughly dry the outside of the re-sealable plastic bag.
4. Complete a chain of custody form.

   Note: Once the outer re-sealable plastic bag is decontaminated, it should not be opened outside of appropriate containment in a laboratory.

5. Place the larger re-sealable plastic bag into an appropriate container for shipping (See Sample Shipment section).

DECONTAMINATION OF BAGS CONTAINING DOCUMENTATION

1. Place sample sheets and other documentation in a separate re-sealable plastic bag. The sheets should be placed two to a bag with the face of each sheet facing out. Securely seal and label the bag (e.g., corresponding sample locations, date and time samples were collected, and name of individual collecting the samples).

   Note: It is important to only write on one side of the paper, the face, when collecting information.
2. Decontaminate the outer surface of the larger re-sealable plastic bag using a fresh pH-adjusted bleach solution (household bleach diluted 1:9; pH-adjusted to 6.8-8.0) with a 10-minute contact time before the re-sealable plastic bag leaves the contaminated area. This solution can be prepared by:

   Step 1: Mixing one part household bleach (5.25 to 6.0 % sodium hypochlorite) with 5 parts water (v/v);
   Step 2: Adding 1 part white vinegar; and
   Step 3: Adding 3 parts of additional water.

   Note: Additional information about decontamination is available at www.epa.gov/opp00001/factsheets/chemicals/bleachfactsheet.htm.
3. Thoroughly dry the outside of the re-sealable plastic bag.
4. Complete a chain of custody form.

   Note: Once the outer re-sealable plastic bag is decontaminated, it should not be opened outside of appropriate containment.
5. Place the larger re-sealable plastic bag into an appropriate container for shipping.

SAMPLE SHIPMENT

1. Transport all samples to the processing laboratory on wet ice or on cold packs.

   Note: Samples may be stored at 2°C–8°C prior to processing and should be processed within 48 hours of collection.

2. Appropriate chain of custody forms and analytical request forms should be included with each shipment sent to the processing laboratory. Containers used to transport the samples and accompanying contaminated documentation and equipment should be prepared and shipped according to the appropriate regulations for transporting infectious. The most current Code of Federal Regulations, International Air Transport Association guidelines, and other appropriate regulator or guidance publications should be consulted for compete instructions. The shipper is responsible for ensuring adherence to the most current and appropriate regulations.

Note: Do not transport contaminated equipment/supplies in the same container as the samples.

Appendix B
Swab and Wipe Sample Interpretation

This Appendix provides technical details of sampling studies to guide the interpretation of data resulting from the use of recommended sampling methods. The data reflect variations in sampling efficiency with bacterial surface coverage, with type of surface, and with variations in the sampling device and other characteristics of the recommended sampling method, given that the realities of any response may dictate some variation from recommended procedures.

Information provided in this appendix is used by technical experts in public health and environmental recovery to inform their consultation with incident command or other authoritative decision makers in the response to a contamination event.

B.1. Swab Sampling Performance

A multi-center validation study involving 12 laboratories was conducted to quantify the performance of a macrofoam swab method using a pure inoculum of *B. anthracis* spores (Hodges 2010). Steel coupons (4-in^2) (26-cm^2) were inoculated with a known concentration of *B. anthracis* spores suspended in 95% ethanol, the inoculum was allowed to dry, and the 26-cm^2 area was sampled with a macrofoam swab pre-moistened with phosphate buffered saline with 0.02% Tween 80 (PBST). To simulate samples with dust and other organisms, some swabs were dipped in a slurry of PBST plus a well characterized dust (A-3, Powder Technology, Inc, Burnsville, MN) before sampling. Laboratories were sent coded swabs in six shipments (three with dust, three without dust). The swabs were processed according to the LRN protocol. Colonies of *B. anthracis* were counted and the numbers compared to the known inoculum level to determine the percent recovery. The results for swabs without dust or other organisms are shown in Table C-1. It should be noted that swabs with dust present yielded recoveries (55,0%, 27.9% and 42.6% for 1, 2, and 4-$\log_{10}$ inocula, respectively), but since the dust content and character will vary from site to site, CDC chose to present the conservative estimate for interpreting contamination on a surface. The macrofoam swabs were pre-moistened with PBST for this study, but in a laboratory comparison, using neutralizing buffer as a pre-moistening liquid was found to result in equivalent recovery efficiency as PBST. Alternate elution buffers may also alter the recovery efficiency of the method; phosphate buffer alone was not as effective at eluting the spores from the swab, though 0.0 5% Tween 20 in buffer was found to be equivalent to 0.02% Tween 80 in buffer.

Table B-1. Recovery Efficiencies Using Macrofoam Swab Sampling and Processing Procedures for *B. anthracis* on Stainless Steel, no dust present.

B. anthracis **Spores/26 cm^2 Area [Average (SD)]**[(a)]	**Number of Swabs**	**Spores Recovered [Average (SD)]**	**% Recovery [Average (SD)]**
49 (7)	118	13 (7)	25.7 (15.2)
506 (86)	120	80 (33)	15.8 (6.6)
41,768 (7415)	116	12,835 (4,392)	31.0 (10.9)
All inoculum levels	354	-	24.2 (13.6)

(a) SD= standard deviation

Using other types of swabs for sampling may affect recovery efficiency. This possibility was evaluated by Rose et al. (2004) who inoculated 10^4 spores of *B. anthracis* Sterne in 95% ethanol onto 26 cm^2 stainless steel coupons. After drying, four types of pre-moistened and dry swabs (cotton, foam, polyester, and rayon) were used to remove the spores and were processed according to the LRN method (Rose 2004,). The recovery efficiencies of four swab materials are shown in Table B-2. Pre-moistened macrofoam and cotton swabs were the most efficient of the four swab types evaluated; pre-moistened polyester and rayon swabs were significantly less efficient. While all swab materials give biased estimates (under-estimates) of surface concentrations, the pre-moistened macrofoam swab is the preferred swab device. It is important to note that sensitivity, specificity, and limit of detection (LOD) have only been established for macrofoam swabs used to sample stainless steel surfaces.

If samplers choose to use another type of swab, pre-moistened cotton swabs have similar recovery efficiency to that of macrofoam swabs, though cotton may contain substances that inhibit PCR reagents, which should be considered if PCR is performed directly on the swab eluent. The differences in recovery efficiency between swab types may be due to differences in the ability of specific swab materials to remove spores from the surface or due to differences in the ability of the spores to be released from the swab during processing.

Table B-2. Recovery Efficiencies of Four Pre-moistened Swab Materials when Sampling Stainless Steel Surfaces

Swab Material	**Cotton**	**Foam**	**Polyester**	**Rayon**
Percent Recovery (SD)	41.7 (14.6)	43.6 (11.1)	9.9 (3.8)	11.5 (7.9)

The LRN method for the macrofoam swab has been validated for smooth non-porous surfaces no greater than 4 in^2 (26 cm^2). Both culture and PCR can be used to determine the presence of spores of *B. anthracis.* When viable spores are present, culture results are provided by the LRN laboratory as "*B. anthracis* spores/cm^2 recovered." A result of "No *B. anthracis* spores detected" should be interpreted in the context of the LOD of this assay (the smallest amount of analyte that can be distinguished from background with 95% confidence), which is 20 spores/26cm^2 (ca. 0.8 spores/cm^2) for stainless steel surfaces. One other caveat needs to be mentioned. Reporting the results as spores/cm^2 assumes that each colony on the agar plate results from the growth of a single spore. In reality, it may result from the growth of a single spore or from a clump of spores. Tween 80 and vortexing are used in the LRN method to help disperse clumps of spores that may be present in the samples, but may not do so completely. PCR is used to confirm *B. anthracis* colonies, and the results are reported as "positive" or "negative." PCR does not differentiate between viable or non-viable spores if performed on the sample directly.

B.2. Wipe Sampling Performance

A multi-center validation study involving 9 laboratories was conducted to quantify the performance of an LRN sponge-stick wipe processing protocol (Rose, 2011). Stainless steel coupons (100 in^2) (645 cm^2) were inoculated with known quantities (26, 528, and 33,140 spores) of *B. anthracis* Sterne spores in 95% ethanol. Seven coupons at each spore concentration were sampled with cellulose sponge-wipes pre-moistened with neutralizing buffer (Sponge-Stick, SSL10NB, 3M St. Paul, MN).

Dust containing a consortia of organisms (A-3, Powder Technology, Inc, Burnsville, MN) were then added to the wipes. A total of 33 wipes were sent to each laboratory in three separate shipments of 11 wipes each. Each shipment consisted of 7 wipes that were used to sample the coupons inoculated with one of the spore quantities, one blank, one dirty blank (background organisms only) and two positive controls (wipes inoculated with the same spore concentration plus background organisms). Upon receipt by the participating laboratory, wipes were stored at 2-8^{o} C until processing. Laboratories processed wipes within 48 hours of sampling according to the LRN protocol. The results are shown in Table B-3. The mean % recovery for all inoculum levels was 29.7% (SD 16.4%).

Table B-3. Recovery Efficiencies using Pre-moistened Sponge-wipes and LRN Processing Procedures for *B. anthracis* on Stainless Steel

***B. anthracis* Spores/645 cm^2 Area [Average (SD)][a]**	**Number of Sponge-wipes**	**Spores Recovered [Average (SD)]**	**% Recovery [Average (SD)}**
26.1 (13.6)	**63**	**9.1 (6.1)**	**32.4. (24.5)**
536.0 (134.1)	**63**	**132.6 (63.0)**	**24.4 (11.2)**
33,140.0 (6,743)	**56**	**9,984.0 (2,707)**	**30.1 (8.2)**

(a) SD= standard deviation

Studies with directly inoculated controls were performed to determine recovery efficiency during wipe processing only. Higher percent recovery for the controls (63.4% (SD 27.5%) vs. 28.9% (SD 16.7%), $p < 0.01$) suggests that a portion of the spores were not removed from the surface with the sponge-stick. The results from sponge-wipes processed by the LRN protocol are given as "*B. anthracis* spores/cm^2 recovered." The results should be interpreted in the context of the LOD of this assay (the smallest amount of analyte that can be distinguished from background with 95% confidence), which is 20 spores/645cm^2 (ca. 0.03 spores/cm^2) for stainless steel surfaces. Reporting the results as spores/cm^2 assumes that each colony on the agar plate results from the growth of a single spore. In reality, it may result from the growth of a single spore or from a clump of spores. Tween 80 and vortexing are used in the LRN method to help disperse clumps of spores that may be present in the samples, but may not do so completely.

Additional evaluations of the validated sponge-wipe protocol were conducted to investigate the effects of validated sponge-wipe protocol with lower spore concentrations and on other surface materials on recovery efficiency, false negative rate, and limit of detection (Krauter 2012). The surrogate spore *Bacillus atrophaeus* var. globigii was employed for these evaluations. The study results show a roughly linear dependence of recovery efficiencies (RE) on surface roughness, where the smoothest surfaces (e.g., stainless steel and ceramic tile) have the higher RE and lower false negative rates. The findings are shown in Table B-4.

Table B-4. Recovery Efficiency and False Negative Rate of Sponge-wipes (averaged over all *B. atrophaeus* spore concentrations) for Each Surface Material with the Corresponding Roughness Index Measurement

	Recovery efficiency, Mean (%)	False negative rate, Mean	Roughness Index (µm)
Stainless steel	48.1	0.1229	0.13
Ceramic tile	48.9	0.1812	0.59
Vinyl tile	25.6	0.2551	1.63
Faux leather	30.3	0.1417	3.27
Painted wood	25.5	0.2000	4.11
Plastic panel	9.8	0.4792	5.88

Both of the above-mentioned sponge – wipe evaluations (Rose 2011, Krauter 2012) were conducted by eluting the spores from the sponge-wipes with phosphate buffered saline (pH 7.2) containing 0.02% Tween 80 using a stomacher. Deviating from the method by using other elution buffers or elution techniques may lead to different recovery efficiencies, sensitivities, specificities, false negative rates and/or limits of detection.

In a limited study, rayon gauze wipes (2” x 2”) pre-moistened with phosphate buffered saline (pH 7.2) containing 0.02% Tween 80, were evaluated as an alternative to Sponge Sticks (Hodges et al 2006b). The mean percent recovery of spores sampled from stainless steel using rayon gauze wipes was 25.4% (SD 18.9%). Thus, pre-moistened gauze wipes may be equivalent to pre-moistened sponge wipes, though validation performance data is not yet available (sensitivity, specificity, reproducibility, precision, LOD).

Appendix C

Non-Validated Sampling Methods

(Adapted from Emanuel et al. 2008)

The Working Group recognizes that an array of sampling methods beyond those described for smooth surfaces in Appendix A may be used in the characterization of a contaminated space, both prior to and after recovery efforts. While these currently non-validated methods may yield information that is more qualitative than quantitative in nature, their application can provide important indicators of the state of a potentially contaminated space. Accordingly they are included here with general instructions to make their application as uniform as possible, in order to help standardize their application.

Use of these methods should be only be considered after consultation with the on-scene response coordinators and participating analytical laboratories, since interpretation of resulting data from these methods may be difficult.

Bulk Sampling

Procedure for Bulk Sampling
The method presented below is for collecting a bulk sample.

Materials and Equipment
The following equipment should be available in order to collect bulk samples:

- Non-powdered sampling gloves
- Disposable or decontaminated spade, spatula, scoop or trowel
- Sterile forceps, scissors, scalpel, or sharp knife
- Sterile sample container of proper size
- Sealable plastic bags
- Documentation materials, digital camera, indelible ink pen, and logbook
- Custody seals, tags, and Sample forms

Procedures

1. Ensure that all of the sample equipment is sterile prior to use.
2. Identify the spot to collect the sample.
3. Collect the sample wearing a pair of non-powdered gloves and document the sample area using a camera and in the logbook.
4. For solids, powders, or granular material, collect the laboratory-specified quantity of the bulk sample with a dedicated sterile spoon, trowel, or spatula and place material into a sterile sample container.
5. For large pieces of material or vegetation that require analysis, discuss with the laboratory the material to be sampled. Large pieces may not fit in the sample container and will need to broken, shaved, cut or chipped into a sterile sample container with dedicated sterile scissors, scalpel, or knife.
6. Place item or pieces of the item in an appropriate sterile sample container.
7. Secure the lid, label and attach the custody seal and triple bag the sample container into sealable bags.
8. Change into new gloves prior to collecting the next sample.
9. Decontaminate outer bag prior to leaving hot zone. This is usually done at the entrance of the personnel decontamination line.

HEPA Vacuum Sampling

Procedure for HEPA Vacuum Sampling on Porous Surfaces

Materials and Equipment

- A portable HEPA vacuum with a nozzle and hose attachment
- Vacuum sample sock assembly with cardboard inlet nozzle
- Power source
- Non-powdered sampling gloves
- Sterile plastic screw-topped sample containers (conical vial or specimen cup)
- Sealable plastic bags
- Small plastic zip tie
- Documentation materials, digital camera, indelible ink pen, and logbook
- Custody seals and tags
- Chain-of-custody forms and shipping paperwork
- Disposable templates to delineate the sample area (optional)
- Isopropyl alcohol wipes

Procedure

1. For each sample collected ensure that a new pair of gloves are worn
2. Determine the location to collect the sample.
3. Wearing a pair of sterile gloves, place a sample template (if using) over the area to be sampled and document the sample area using a camera, and drawing a map in the logbook.
4. Place the cardboard vacuum filter sock inlet assembly securely into the vacuum hose nozzle.
5. With the vacuum on, place cardboard nozzle on the surface to be sampled and vacuum designated area using an overlapping 'S' pattern both in the horizontal and vertical directions. Collect the sample in an area up to several square feet at a rate of 3 – 5 seconds per foot.
6. Once the sample has been collected, turn off the vacuum and remove the cardboard filter sock inlet assembly from the vacuum nozzle.
7. Touching only the blue portion; remove the filter sock from the assembly tube, and zip tie the blue portion of the bag closed. Then place sock into a sterile sample container.
8. Secure the lid, label and attach the custody seal and triple bag the sample container into a sealable bag.
9. To prepare for the next sample, with the vacuum off, wipe the first several inches of the inside and outside of the vacuum nozzle with an isopropyl alcohol wipe and cover with a clean sample glove.
10. Change out the used gloves with new gloves prior to collecting the next sample.
11. Decontaminate outer sample bag prior to leaving hot zone. This is usually done at the entrance of the personnel decontamination line.
12. Package samples for transport.

13. Fill out Chain-of-Custody form, and make a copy.
14. Secure samples in shipping container with Chain-of-Custody and attach Custody seals.
15. Fill out shipping manifest or contact courier.

Air Filter Samples

Procedures for Air Filter Sampling

Below is procedure for collecting air samples with an open-face filter cassette.

Materials and Equipment

- Calibrated personal sampling pump
- Rotameter (air flow meter) or dry cell calibrator
- 3-piece, 37-milimeter (mm) cassette preloaded with sterile 0.45 micron mixed cellulose ester (MCE), Gelatin or Teflon (PFTE) sample filter
- Flexible Tygon™ tubing
- Sterile tweezers
- Sterile non-powdered sampling gloves
- Cassette opening tool
- Sealable plastic bags
- Documentation materials, digital camera, indelible ink pen, and logbook
- Custody seals and tags
- Chain-of-custody forms and shipping paperwork

Procedure

1. To calibrate the sampling pump, take a 3-piece cassette with a preloaded filter and remove the inlet and outlet plugs. Connect flexible Tygon™ tubing from the inlet of the filter cassette to the outlet of the calibrator. Train by attaching one end of the Tygon™ tubing to the inlet of a dedicated open-faced. Attach another piece of Tygon™ tubing from the outlet of the filter cassette to the pump manifold.
2. Calibrate the pump flow rate to the rate specified by the method: greater than 2.5 liter per minute (LPM) for MCE or Teflon filters and 2.0 LPM for the gelatin filters. If using a rotameter for calibration, then it should be calibrated with a primary standard such as the dry cell calibrator before using. Rotameters are considered secondary standards.
3. When calibration has been completed, remove the filter cassette, cap the inlet and outlet with the plugs, and save the filter cassette for recalibration at the end of sampling. Record initial flow rate of the sample pump from the calibration in the logbook and on the Cain-of-Custody form. The flow rate is used to calculate the airborne concentration of the contaminant.
4. Don sterile non-powdered sampling gloves.
5. To prepare the open-faced cassette for sampling, utilize the cassette opening tool to remove the inlet section of the 3-stage cassette, leaving the other two sections in place.
6. Remove the outlet end plug and attach one end of the flexible Tygon™ tubing to the outlet of the cassette and the other to the pump.

7. Place the sample cassette and the pump in the desired location and photo-document and map the location.
8. Turn on the pump and record the time. Be sure the sampling cassette is oriented at a 45 degree angle downward. This prevents large particles from being collected that otherwise would not be collected.
9. Document the location and the sample location using a camera, drawing a map, and recording notes in the logbook.
10. Once the sample has run for the specified amount of time, remove the cassette and replace the inlet stage and the outlet and inlet plugs. It is important to note that with gelatin filter sampling times should not exceed 30 minutes since the gelatin can dry out.
11. Triple-bag the sample filter cassette in sealable plastic bags.
12. Check the final flow rate of the sampling train. Place the calibration cassette in the sample train and check with a rotameter or a dry cell calibrator the final flow rate just as in the initial calibration. Record this value in the sample form, on the Chain-of-Custody, and in the logbook.
13. Pre and post sampling train calibration can be done either inside or outside the hot zone. For calibration outside the hot zone the sampling equipment must be protected from contamination or easily decontaminated. Otherwise, pre and post sampling train calibration should be done in the hot zone.
14. Label and attach a custody seal to the cassette.
15. Decontaminate the outer sample bag prior to leaving hot zone. This is usually done at the entrance of the personnel decontamination line.
16. Package samples for transport.
17. Complete the Chain-of-Custody form and any other paperwork and make a copy.
18. Secure samples in shipping container with Chain-of-Custody and attach Custody seals.
19. Fill out shipping manifest or contact courier.

Air Impactor Samples

Procedures for Impactor Air Sampling

Below is procedure for collecting air impactor samples with petri dishes specific to the contaminant being sampled.

Materials and Equipment

- Calibrated high-flow sampling pump (28.3 LPM)
- Rotameter (air flow meter) or dry cell calibrator
- Calibration adapter for impactors
- Sterile single or six stage impactor
- Sterile Petri dish and agent-specific agar for each stage
- Flexible Tygon™ tubing
- Sterile non-powdered sampling gloves
- Sealable plastic bags
- Parafilm M® wax strips
- Sample labels and wax pencil
- Documentation materials, digital camera, indelible ink pen, and logbook
- Custody seals and tags
- Chain-of-custody forms and shipping paperwork

Procedure

1. For each sample collected, ensure that a new pair of sterile gloves is worn.
2. Set the pump flow rate to 28.3 LPM per minute or as specified in the analytical method, and turn it on.
3. To calibrate the impactor, aseptically remove the lids from the calibration set of Petri dish(es) and keep lids in a clean sealable plastic bag. For the single stage impactor, place each one calibration Petri dish on the stage and reassemble the impactor. For the 6 stage impactor, place one of the calibration Petri dishes on each of the impactor stages and reassemble the stages in the correct numerical order. Attach the calibration adapter to the top of the impactor. Attach flexible Tygon™ tubing from the impactor calibration adapter to the calibrator or rotameter inlet. Attach the second piece of tubing from the outlet of the impactor to the inlet of the sample pump. Turn on the calibrator and record the initial flow rate in the logbook.
4. Calibration of the sampling train can be performed outside the hot zone such as in the sample preparation area. If using a rotameter for calibration, then it should be calibrated with a primary standard such as the dry cell calibrator. Rotameters are considered secondary standards.
5. After calibration, remove the calibration Petri dishes from each stage of the impactor and cover with a lid. These can be reused for calibration several times until they begin to dry out and not more than one day.
6. In preparation to sample, aseptically remove lids from the sample Petri dish(es) and keep in a clean sealable plastic bag. For the single stage impactor, place one Petri dish on the stage and reassemble the impactor. For the 6 stage impactor, place on of the 6 Petri

dishes on each impactor stage and reassemble the impactor ensuring that the stages are in the correct numerical order. Connect the Tygon™ from the outlet of the impactor to the inlet of the pump.

7. Place the impactor and pump in desired sample location and photo document and map the location.
8. Start the pump and record the time sampling began and the time the sampling is completed. Sampling times should be between 10 to 15 minutes. At completion of sample time, don sterile gloves and aseptically remove the petri dish(es), cover with lids and seal each dish with Parafilm M® to secure, label each dish with the wax pencil including the stage number and place into sterile zippered sample bag upside down (agar oriented up).
9. Double bag each sample.
10. Decontaminate outer bag prior to leaving hot zone. This is usually done at the entrance of the personnel decontamination line.
11. For post sampling calibration, aseptically remove lids from each of the pre-calibration sample Petri dishes and place on the impactor stages. Attach the tubing to the calibrator and the pump as in the initial calibration.
12. Turn on pump and record the post sampling flow rate in the log book. Pre and post calibration flow rates are very important in determining final contaminate concentration.
13. Pre and post sampling train calibration can be done either inside or outside the hot zone. For calibration outside the hot zone the sampling equipment must be protected from contamination or easily decontaminated. Otherwise, pre and post sampling train calibration should be done in the hot zone.
14. Package samples for transport.
15. Fill out Chain-of-custody form, and make a copy.
16. Refrigerate samples or package with ice, ensuring agar does not freeze.
17. Secure samples in shipping container with Chain-of-custody and attach Custody seals.
18. Fill out shipping manifest or contact courier.
19. Prior to use to collect another sample, the impactor must be autoclaved.

Impinger (Wet Method) Air Samples

Procedures for Impinger Air Sampling

Below is procedure for collecting air samples with an impinger using a wet method.

Materials and Equipment

- High Flow Sampling Pump
- Dry cell calibrator and stand
- Two sterile impinger, pump attachment, and sterile impinger fluid
- Teflon or Parafilm M® tape
- Flexible tygon tubing
- Sterile sample container bottle
- Sterile non-powdered sample gloves
- Documentation materials, digital camera and logbook
- Custody seals, sealable plastic bags, and tags
- Sample labels, documentation forms, permanent marker(s)
- Chain-of-custody forms and shipping paperwork

Procedure

1. For each sample collected, ensure that a new pair of sterile gloves is worn.
2. Aseptically fill an impinger with appropriate sterile fluid and attach to pump. This should be done outside the hot zone in a clean area.
3. Set up the sampling train by attaching Tygon™ tubing to outlet of impinger and the other end to inlet of the sample pump.
4. In a clean area, calibrate the sample train by attaching another piece of Tygon ™ tubing to the outlet of the impinger and the other end to a rotameter or dry cell calibrator. Adjust pump to the desired flow rate of 12.5 LPM. If using a rotameter for calibration, then it should be calibrated with a primary standard such as the dry cell calibrator before using. Rotameters are considered secondary standards.
5. After pre-sampling calibration, remove impinger, place caps or Parafilm M® over both the inlet and outlet of the impinger and set aside to use to check the flow rate after the sample is collected.
6. Don a new pair of sterile gloves and attach a second sterile impinger, filled with appropriate sterile fluid, to the sampling train.
7. Place sampling train in desired sample location and turn on pump.
8. Photo document sample location, draw map and record sample start time in the log book.
9. After sampling time has elapsed, turn off pump, don sterile gloves and aseptically remove the impinger.
10. Ascetically transfer impinger fluid to sample container bottle can be done either inside or outside the hot zone. If done outside the hot zone, place a cap or Parafilm M® over the inlet and outlet of the impinger. It is important to keep impingers upright to prevent loss of fluid due to leaking or spillage. Fluid transfer done outside the hot zone must be done in an appropriate fume hood. If impinger fluid will be transferred to sample container bottle in the hot zone, don sterile gloves and aseptically remove the impinger, transfer

fluid to labeled, sterile sample container and seal the lid with Teflon or Parafilm M® tape.

11. Double bag the sample.
12. For post sampling train calibration, don sterile gloves and attach a fluid filled calibration impinger to the sample train as described in Step 4. Turn on pump and record flow rate. Record flow rate in log book.
13. Pre and post sampling train calibration can be done either inside or outside the hot zone. For calibration outside the hot zone the sampling equipment must have be protected from contamination or easily decontaminated. Otherwise, pre and post sampling train calibration should be done in the hot zone.
14. Decontaminate sample bag before leaving hot zone. This is usually done at the entrance of the personnel decontamination line.
15. Package samples for shipment including ice, if needed.
16. Complete Chain-of-custody form and place in sample shipment container.
17. Secure shipment container and complete shipping manifest.
18. Prior to another use, the impinger used to collect the sample must be autoclaved.

Liquid Sampling for Biological Agents

Note: For drinking water samples please refer to the sample note at bottom of procedures

Materials and Equipment

- Non-powdered sample gloves
- Plastic or glass 1-liter sample bottle
- Bacon bomb sampler, Kemmerer sampler, Dip sampler, Bailer, or large 100 ml disposable syringe.
- Sealable plastic bags
- Parafilm M® wax strips or
- Teflon tape
- Sample labels and wax pencil
- Documentation materials, digital camera, indelible ink pen, and logbook
- Custody seals and tags
- Chain-of-custody forms and shipping paperwork
- Shipping Manifest

Procedure

1. For each sample collected, ensure a new pair of sterile gloves is worn
2. Ensure all equipment is sterilized or decontaminated prior to use
3. Select appropriate apparatus based on sample depth and locations. For example, in small puddles the syringe may be the best apparatus to use.
4. If a sample collection device was used, transfer the sample to an appropriate sized sterile plastic or glass container
5. To collect the sample directly into a bottle, remove bottle lid and protect from contamination by placing in new sealable plastic bag. Grasp bottle at the base with one hand and plunge bottle mouth down into the water to avoid introducing surface scum. For large sample volumes, the above approach may not be possible; therefore, a sterile transfer container may be needed.
6. If water body is deep and is static, an artificial current can be created, by moving bottle horizontally in the direction it is pointed and away from sampler collector
7. Tip bottle slightly upwards to allow air to exit and the bottle to fill
8. Pour out a small portion of the sample to allow an air space of 2.5-5 cm (1"-2") above each sample for proper mixing of sample before analyses
9. Cap the bottle and seal lid with Parafilm M®, Teflon tape or equivalent
10. Label samples.
11. Photo document sample at the sample location.
12. Double bag sample.
13. Decontaminate outer bag, if needed, in the hot zone.
14. Package sample(s) for shipment, including ice to keep sample cool.
15. Complete Chain-of-custody form and place in sample shipment container.
16. Secure shipment container and complete shipping manifest.

* U.S. EPA. 2011 Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=238310&fed_org_id=1253&address=nhsrc/si/&view=desc&sortBy=pubDateYear&showCriteria=1&count=25&searchall='water%20security'%20AND%20'biological'

Surface Soil Sampling for Biological Agents

Materials and Equipment

- Non-powdered sample gloves
- Sterile stainless steel or plastic scoop or trowel
- Sterile 250 ml sample jar
- Sealable plastic bags
- Chain-of-custody forms and custody seals
- Sample labels, sample documentation form, permanent marker(s)
- Shipping Manifest

Procedure

1. For each sample collected, ensure a new pair of gloves is worn
2. Ensure all equipment is sterilized or decontaminated prior to use
3. Using a scoop or trowel, collect 50-100 g of soil from desired location (fill the container)
4. If possible remove rocks, vegetative matter, or sharp objects from soil
5. Place sample in appropriate sterile plastic container
6. Cap container with the sample jar lid
7. Label samples.
8. Photo document sample at the sample location, draw a map and log information in the logbook.
9. Double bag sample.
10. Decontaminate outer bag, if needed, in the hot zone.
11. Package sample(s) for shipment, including ice to keep sample cool.
12. Complete Chain-of-custody form and place in sample shipment container.
13. Secure shipment container and complete shipping manifest.

Appendix D
Documentation and Decision Support Tools

Several different software tools have been designed to manage and document data from sample collection as well as assist in developing grid and statistical sampling plans. Managing the data collected as part of a *consequence management* effort is very important. To ensure the integrity of sample results, various types of documentation need to be completed throughout the sampling process (i.e., from sample collection through sample analysis). The use of decision support tools can support the development of a sampling plan based on statistical sampling theory and the statistical analysis of sample results to support confident decision-making. It is critical to ensure that the information gathered can be easily and quickly shared among the various state, local, and federal agencies.

Since the collection of samples may be associated with a crime scene or suspicion of a criminal incident, the resulting information, the approach utilized to develop the sampling plan, and manner in which samples were collected will be factored into the usability of those samples in a court of law, as well as to ensure process quality. Sampling teams need to be aware that samples collected may be or become criminal evidence, and certain additional procedures associated with sample collection will be necessary.

D. 1 Sample Documentation

In order to provide accurate and high-quality information, it is important for sampling personnel to understand not only what needs to be documented, but also why it needs to be documented (Emanuel et al. 2008). To meet laboratory submission requirements, and to interpret sampling results, information about the sampling process must be documented and should include information about general site details as well as specific information about individual samples. Much of the needed general site information is documented in the sampling plan, described earlier in Section 4.4. Additional documentation and information can also be found in the Incident Action Plan (IAP). The IAP is developed by the IC/UC and describes tactical objectives and support activities for one operational period, generally 12 to 24 hours.

Information that should be documented includes:

- Procedures for collecting and processing the samples;
- Description of the items that may be sampled (e.g., desk, carpet, wallboard, etc.);
- Description of the location where the sample was collected and associated sample number (e.g., Room 110, sample collected from on top of file cabinet in North, East corner and sample number is 1011). This is very important so that a sample result can be associated to a specific item sampled and its location.
- Description of surfaces that must be sampled (e.g., porous, non-porous, rough, smooth, etc.) and surface materials or coating (e.g., plastic, metal, painted surface, etc.);
- Weather conditions, including temperature and wind.

To meet the documentation needs for individual samples, sample collection, sample analysis request forms, and chain of custody forms should be completed. The information requirements in these forms are described in the next sections. Additional information that may also be important to document in sampler field notes includes:

- Notes regarding the sampling process that might be of interest to future analysis of the data (e.g., surface was noticeably contaminated with particulate material)
- The area that was sampled (e.g., a swab sample using a template with an area of 100 cm^2)
- Information about the photographs taken
- Document the method used to establish the location (e.g., measured with a tape measure, laser positioning system, GPS, manual location on a map, etc.)

The use of handheld data collection devices like a personal data assistant (PDA) improves the quality of the field data collected. A software program can be used to collect information for sample documentation. These programs offer a wide flexibility in managing the information electronically.

- A unique sample identification number, date, and time for each sample
- A detailed description of the matrices that was sampled (e.g., water, air, soil, solids, etc.)
- A COC form must be produced before samples can be transported from the site, and must have a signature confirming the collection and release of the samples
- Establishing a datum for sampling location identification (e.g., the origin location for a local coordinate system, UTM (Universal Transverse Mercator) coordinates, latitude/longitude, etc.)
- Sampling location, in X, Y, Z space relative to the origin location

D.1.1 Sample Collection Form

During sample collection in the hot zone, individual sample information should be recorded. However, recordkeeping should be kept to the minimum necessary as any documentation will have to be decontaminated upon leaving the contaminated area. The sample collection form serves as the documentation for the sampling incident. First, each sample should be given a unique sample identification number. In addition to the unique identification number, the following information should be recorded on the form:

- The date and time collected for each sample
- The type of sample (e.g., surface, air, and bulk, etc.)
- The type of sample collection technique (e.g., swab, wipe, sock vacuum, etc.)
- The orientation of the surface (e.g., horizontal, upwards, vertical, etc.)
- The surface area sampled (e.g., square centimeters wiped)

- If desired, indicate whether the sample was prescribed as part of a probability-based sampling design, a judgmental sample, or other
- Documentation of sampler's name
- Other sample location information of note (e.g., on what floor the sample was collected, room number, area identifier, etc.)

D.1.2 Sample Analysis Submission Form

After sampling is completed, a sample analysis submission form should be finished and submitted to the LRN along with the samples. Sampling personnel should meet with or contact their local LRN to obtain this form. While individual LRN laboratories may have different required fields, the following information is included in any form:

- Submitter information
- Specimen type, suspect organism, and source
- Date and time collected
- Analytical processing request

D.1.3 Chain of Custody (COC) Form

A COC form documents transfer of sample custody from one individual to another, from the time the sample is collected until final analytical disposition. Each individual in possession of the sample must be noted by recording his or her signature on the form. The COC record should include instructions for the laboratory technician as to analytical methods, potential dangers, and any pertinent handling procedures that should be observed. The COC record must include at least the following information:

- All available information regarding the potential hazards associated with the agent
- Handling procedures associated with the samples
- Sample identification number
- Sample concentration, if known
- Sampling location
- Collection date and time
- Sample matrix
- Names and signatures of the samplers
- Signatures of all individuals who had custody of the samples (EPA 2006a)

An unbroken COC must be maintained for all samples from collection through analysis and archiving. In order to maintain COC, the form must be readily accessible when transferring samples from one individual to another. Therefore, COC forms should not be placed inside the primary sample containment. A copy of the record will be kept with the samples until they are analyzed and returned with the analytical results or will be maintained on site at the laboratory if samples are archived for later use or collection by law enforcement.

D.2 Data Management Plan

The Data Management Plan (DMP) establishes an overall plan for the data management requirements for a specific project. The purpose of the DMP is to provide the necessary management, control necessary sample nomenclature, maintain quality control information, Geographic Information System (GIS) information, and control and inventory of all data.

The primary functions of the DMP are as follows:

- record keeping
- data quality control
- storage and retrieval systems
- handling of classified data
- planning, scheduling, and delivery of data

D.2.1 Managing Large Amounts of Data

In managing large amounts of data, the decision makers should pre-plan their data management requirements. With large amounts of data, serious consideration should be given to utilizing a formal database structure for saving and querying data. Databases preserve relationships between sample data. Spreadsheet applications do not provide the same rigor of preservation of sample data attributes. In addition, spreadsheet applications may be too cumbersome to manage large data sets. With more comprehensive database structures, not only will the data associated with sample locations and analysis results be archived, but other spatial information such as facility maps and spatial mapping of results may be maintained as well.

Another consideration for managing large amounts of data is security. With a secure database, access can be managed through login privileges granted by the decision makers thereby limiting access to the data to those with a need-to-know. Data sharing can be managed with a more sophisticated database engine. There can also be allowances to limit which personnel have permission to modify data in the database, in order to preserve integrity of the data. For instance, a secure database will be critical in managing data associated with a terrorist threat response.

The staff that maintains the database should be aware of the structure of the database and the master key identifiers used to manage the data. There is a possibility for corruption of a database if the relationships are not maintained appropriately in the database. Care should be taken to maintain these relationships.

D.2.2 Electronic Data Format

Whenever possible, data should be stored in an electronic database. There may be a need to export data from a database to a spreadsheet application in order to analyze the data with other tools (e.g., Excel, mapping software, etc.). There may also be a need to establish protocols for saving spatial information, such as CAD drawings, GIS maps, bitmap images, etc. Photographic documentation should also be considered in the specifications for electronic data storage and capture.

D.3 Decision Support Tools

If possible, the use of decision support tools throughout the response phases is recommended to help facilitate the design of a sampling plan. Decision support tools may be used to codify the processes for developing a sampling plan and to document the data and assumptions associated with the plan. These tools should facilitate better defensibility of the assumptions, goals and data associated with the project.

Decision support tools provide users with the following capabilities to:

- Develop DQOs
- Develop defensible sampling design plans (e.g., locating hotspots, testing hypotheses of the confidence in meeting a cleanup goal, etc.)
- Provide sampling locations via spatial representation
- Display building or site layout (e.g., engineering drawings)
- Document information associated with sample collection (e.g., sample collection method, location, surface type, sampling ID number, etc.), including electronic data capture with handheld devices
- Provide sample analyses results via spatial mapping
- Analyze data to determine statistical relationships and information suitable for decision making
- Optimize sampling design if an adaptive sampling strategy is desired

Appendix E

Details on Application of Combined Judgmental and Random (CJR) Sampling Approach

For the CJR sampling approach, several input parameters affect the required number of probabilistic samples. These include 1) the percent confidence (X%) desired, 2) the minimum percent (Y%) of the decision area stated to not contain detectable contamination, 3) the number of judgmental samples taken, 4) how much more likely it is a judgmental sample location contains detectable contamination than a probabilistic sample location, and 5) the expected *a priori* probability a judgmental sample will detect contamination. These parameters are discussed and guidance for selecting them is provided after the next paragraph.

An important assumption of the mathematical model used in the CJR approach is that the decision area can be divided into areas of "high" and "low" probabilities of being contaminated (the high probability areas need not be contiguous, and the same for low probability areas). The CJR model assumes all of the high probability areas are sampled judgmentally. In essence, the judgmental sample locations define the high probability areas in the sampling plan. Consequently, fewer probabilistic samples are necessary when more judgmental samples are taken and/or when locations with judgmental samples are more likely to contain detectable contamination. Fewer probabilistic samples are also necessary as the *a priori probability* that a judgmental sample will detect contamination increases.

The key parameters for the CJR sampling approach are briefly explained below with guidance on how to specify a value for each parameter.

- *Desired confidence (X%)*: The CJR sampling approach provides for stating that there is X% confidence that at least Y% of a decision area has no detectable contamination if all of the judgment and random samples obtained are non-detects. There is precedence in environmental regulations for specifying X = 95, but less or greater confidence may be deemed appropriate depending on risk/consequence evaluations.
- *Minimum percent (Y%) of the decision area that can be stated to not contain detectable contamination*: Ideally Y% would be 100%, but that would require sampling 100% of the decision area. When choosing the Y% parameter, the team must balance between resources/cost and risks/uncertainty. Higher Y% values require more samples. Often Y% will range between 90% and 99.5% with 99% often used.
- *Number of Judgment Samples*: The number of judgment samples taken will be determined using expert judgment, knowledge of the event, and previous experience. Judgment samples should be obtained from all areas where contamination is deemed to be most likely.
- *How much more likely it is that a judgment sample location contains detectable contamination than an uninformed random sample*: It is recommended that this parameter be between 1 and 3, unless there is significant evidence that it is higher. This parameter may be derived from expert opinion, knowledge of the event and/or experience from previous studies. If the value of 1 is chosen for this parameter, equal weight is given to the judgment and random samples.

- *Expected a priori probability (p) that a judgmental sample will contain no detectable contamination:* This parameter is derived from expert opinion and/or results from previous sampling in the decision area. If the CJR sampling approach is being applied after decontaminating the area, information from previous studies regarding the effectiveness of the decontamination process may be used. Where proven, highly effective decontamination technologies are applied, the *a priori* probability might be quite high (0.90 to 0.99). If little is known about the possibility of contamination in the area, an uninformed *a priori* probability of 0.50 is used.

In dealing with contamination incidents, subject matter experts may recommend values of the above parameters, but the ultimate decision is with the IC/UC.

The CJR approach has been incorporated into the freely available Visual Sample Plan (VSP) software (VSP Implementation Team 2010, Matzke et al. 2010) that calculates the required number of random samples given the number of judgmental samples and other parameter values. The VSP software provides for selecting both judgment and random sample locations within the facility. Random samples can be placed either completely at random or using a systematic grid sampling scheme with a random start for the grid. The systematic grid will better protect against a large unsampled area where a large "hotspot" could go undetected. The number of random samples required also depends on the number of possible unique sample locations in the population. For a large facility the number of possible area samples (e.g., 10 cm x 10 cm wipes) can be very large. If a 3-dimensional representation of the facility is constructed in VSP, the number of possible area samples for the surfaces of interest is determined automatically and incorporated in the sample requirements calculations.

Appendix F
Example of a Site-Specific Sampling Plan

Note: This plan was developed as part of an EPA biological remediation demonstration known as Bio-Response Operational Testing and Evaluation (BOTE II).

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF ENVIRONMENTAL CLEANUP
EMERGENCY RESPONSE UNIT

Site Specific Sampling Plan

Project Name: ___BOTE Phase 2_________ Site ID: ______

Author: _________ Company:___________________. Date Completed: ___

This Site Specific Sampling Plan (SSSP) is prepared and used in conjunction with the Quality Assurance Plan (QAP) for the Emergency Response Unit for collecting samples during this Removal Program project. The information contained herein is based on the information available at the time of preparation. As better information becomes available, this SSSP will be adjusted.

When inadequate time is available for preparing the SSSP in advance of the sampling event, a Field Sampling Form may be prepared on-site immediately prior to sampling. This full length version of the SSSP is written after the sampling event and the completed Field Sampling Form attached to it.

1. Approvals

Name, Title	Telephone, Email, Address	Signature
On-Scene Coordinator		
ERU Quality Assurance Coordinator		

I. Project Management and Organization

2. Personnel and Roles involved in the project:

Name	Telephone, Email, Company, Address	Project Role	Data Recipient
		On Scene Coordinator	Yes
		Author of SSSP, START Project Manager	Yes

		ERU Quality Assurance Coordinator	No
		START Quality Assurance Reviewer	Yes
		Sampling Leader	Yes

3. Physical Description and Site Contact Information:

Site Name	BOTE Phase 2	
Site Location	See Figure 1	
Property Size	See Figure 1	
Site Contact		**Phone Number:**
Nearest Residents		**Direction:**
Primary Land Uses Surrounding the Site	Commercial, university	

4. The proposed schedule of project work follows:

Activity	**Estimated Start Date**	**Estimated Completion Date**	**Comments**
SSSP Review/Approval			
Mobilize to / Demobilize from Site			
Sample Collection			
Laboratory Sample Receipt			Saturday receipt may be requested
Laboratory Analysis			
Data Validation			

5. Historical and Background Information

Describe briefly what you know about the site that is relevant to sampling and analysis for this investigation.

On Wednesday, September 7,2011, a 56 year-old female is admitted to the Eastern Idaho Regional Medical Center (EIRMC) in Idaho Falls, Idaho after experiencing vomiting, confusion, incoherent speech and a severe headache for the past 6 hours. On September 9, the LRN laboratory confirms *B. anthracis* by Polymerase Chain Reaction (PCR) and from a culture of the cerebrospinal fluid. She dies from inhalation anthrax on September 10, 3 days after admission.

On Thursday, September 8,2011, a 64 year-old male is admitted to the Portneuf Medical Center (PMC) in Pocatello, ID with symptoms suggestive of pneumonia. His illness began on September 6. As of September 10, the patient remains hospitalized with inhalation anthrax and is being treated intravenously with several antibiotics.

On Saturday, September 10,2011, an epidemiological investigation links both patients to the same place of employment in Idaho Falls. The deceased woman, Ann Halation, was identified as the secretary to the President of Southeastern University. Her office is located in the Administration Building on the university campus. The hospitalized male, Austin Powders, was determined to be a janitor employed at the same building.

6. Conceptual Site Model

Example: Contaminant: Mercury
Transport Mechanism: vapor moving on air currents
Receptors: people living in the house

Contaminants: B. anthracis

Transport Mechanisms: Moving on air currents, on surfaces in the building

Receptors: people through inhalation or direct contact of spores

7. Decision Statement

Examples: 1) Determine whether surface contamination exceeds the established action level;
2) Determine appropriate disposal options for contaminated materials.

The decision(s) to be made from this investigation is/are to:

1) Determine extent of contamination within building.
2) Determine the appropriate decontamination method for the building and related items.
3) Determine if contamination is contained to the building or not.

8. Action Level

State the analyte, concentration, and units for each selected action level. Describe the rationale for choosing each action level and its source (i.e. MTCA, PRG, ATSDR, etc.) Example: The action level for total mercury in soil is 6.7 mg/kg (from Regional Screening Level residential).

The Action Level is being determined by the UC.

II. Data Acquisition and Measurement Objectives

9. Site Diagram and Sampling Areas

A Sampling Area is an area within in which a specific action will be performed.
Examples : 1) Each drum on the site is a Sampling Area;
2) Each section of sidewalk in front of the residence is a Sampling Area;
3) Each sampling grid section is a Sampling Area.

Figure 1, 2, and 3 display the site.
Each room is a decision area.
The outdoors is a decision area.

10. The Decision Rules

These can be written as logical If…, Then.. statements. Describe how the decisions will be made and how to address results falling within the error range of the action level. Examples: 1) In the Old Furnace Sampling Area, the soil in the area around the furnace structure will be excavated until sample analysis with XRF shows no mercury concentrations in surface soil above the lower limit of the error associated with the action level, 18.4 mg/kg. 2) If the concentrations of contaminants in a SA are less than the lower limit of the error associated with the action level, then the area may be characterized as not posing an unacceptable risk to human health or the environment and may be dismissed from additional RP activities. The area may be referred to other Federal, State or Local government agencies.

The following statement(s) describe the decision rules to apply to this investigation:
To be determined by the UC with the help of the TWG. Sample results will be utilized by the TWG to recommend a decon method for the building

11. Information Needed for the Decision Rule

What information needs to be collected to make the decisions – this includes non-sampling info as well: action levels, climate history, direction of water flow, etc. Examples: Current and future on-site and off-site land use; wind direction, humidity and ambient temperature; contaminant concentrations in surface soil.

The following inputs to the decision are necessary to interpret the analytical results:

Action Levels, extent of contamination (areas and concentration), cost effectiveness of each decon technology, dispersal method, status of HVAC system since attack

12. Sampling and Analysis

For each SA, describe:

1. *sampling pattern (random, targeted, scheme for composite)*
2. *number of samples, how many to be collected from where, and why*
3. *sample type (grab, composite)*
4. *matrix (air, water, soil)*
5. *analytes and analytical methods*
6. *name and locations of off-site laboratories, if applicable.*

Non-Impact Rooms: Evaluate if contamination has been tracked into rooms that are not believed to have been impacted by directly by source the source letter.   Tracking could be by foot traffic or fomite (i.e. cross contaminated mail, personal belongings). Also, to determine if contamination passed through the HVAC system and impacted the room.

- 1 discrete sample at entry on the floor
- 1 horizontal composite sample (discretionary) floor or surface sample
- 1 ceiling vent sample

- 1 optional discretionary sample (i.e. electro-static surface, mail drop areas, etc)

Total rooms: 23 Total samples: Up to 92

"NIOSH " Rooms: More fully characterize rooms that have had limited sampling from the NIOSH investigation.

- 1 discrete sample at entry on floor
- 1 optional discretionary sample (i.e. electro-static surface, mail drop areas, etc)

Total rooms: 6 Total samples: Up to 12

2nd Floor Exclusion Zone: Provide quantitative analysis of high-spore load areas to potentially support decontamination planning efforts and estimate spore size distribution.

- 2 six-stage impactor samples (Rooms 201 and 201A)
- 2 quantitative discrete samples in each room (discretionary)
- 1 vertical sample per room

Total rooms: 4 Total samples: Up to 24

Hallways / Stairs: To determine if contamination was tracked via movement from source areas.

- One 4-point composite of the upper hallway
- Once HVAC return register
- One 3-point composite from each stairwell

Total areas: 3 Total samples: Up to 4

Outside Evaluation: To determine if contamination has been tracked away from the building

- 4 discretionary discrete samples from concrete areas

Total areas: 1 Total samples: Up to 4

RV-PCR Evaluation: To provide samples to evaluate EPA's rapid viability PCR

Total samples: 12

Field Blanks: Handle samples in the field without collection to support Quality Assurance

Total samples: Up to 15

13. Applicability of Data (place an X in front of the data categories needed, explain with comments)

Do the decisions to be made from the data require that the analytical data be:

1) definitive data, 2) screening data (with definitive confirmation) or 3) screening data (without definitive confirmation)?

*X___**A) Definitive data** is analytical data of sufficient quality for final decision-making. To produce definitive data on-site or off-site, the field or lab analysis will have passed full Quality Control (QC) requirements (continuing calibration checks, Method Detection Limit (MDL) study, field duplicate samples, field blank, matrix spikes, lab duplicate samples, and other method-specific QC such as surrogates) AND the analyst will have passed a Precision and Recovery (PAR) study AND the instrument will have a valid Performance Evaluation sample on file. This category of data is suitable for: **1) enforcement purposes, 2) determination of extent of contamination, 3) disposal, 4) RP verification or 5) cleanup confirmation.***

Comments:

*___**B) Screening data with definitive confirmation** is analytical data that may be used **to support preliminary or intermediate decision-making** until confirmed by definitive data. However, even after confirmation, this data is often not as precise as definitive data. To produce this category of data, the analyst will have passed a PAR study to determine analytical error AND 10% of the samples are split and analyzed by a method that produced definitive data with a minimum of three samples above the action level and three samples below it.*

Comments:

*___**C) Screening data** is analytical data which has not been confirmed by definitive data. The QC requirements are limited to an MDL study and continuing calibration checks. This data can be used for making decisions: **1) in emergencies, 2) for health and safety screening, 3) to supplement other analytical data, 4) to determine where to collect samples, 5) for waste profiling, and 6) for preliminary identification of pollutants**. This data is not of sufficient quality for final decision-making.*

Comments

14. Special Sampling or Analysis Directions

Describe any special directions for the planned sampling and analysis such as additional quality controls or sample preparation issues. Examples: 1) XRF and Lumex for sediment will be calibrated before each day of use and checked with a second source standard. 2) A field blank will be analyzed with each calibration to confirm the concentration of non-detection. 3) A Method Detection Limit determination will be performed prior to the start of analysis so that the lower quantitation limit can be determined. 4) If particle size is too large for accurate analyses, the samples will be ground prior to analysis. If the sample contains too much moisture for accurate analyses, the sample will be decanted and air dried prior to analysis.

N/A

15. Method Requirements

[Describe the restrictions to be considered in choosing an analytical method due to the need to meet specific regulations, policies, ARARs, and other analytical needs. Examples: 1) Methods must meet USEPA Drinking Water Program requirements. 2) Methods must achieve lower quantitation limits of less than 1/10 the action levels.3) Methods must be performed exactly as written without modification by the analytical laboratory.]

Only CDC approved methods will be used for sampling and analysis.

16. Sample Collection Information

[Describe any activities that will be performed related to sample collection]

The applicable sample collection Standard Operating Procedures (SOPs) or methods will be followed and include: Field Activity Logbook SOP Sample Packaging and Shipping SOP Instrument SOPs: Other SOPs: Attachment A: CST Sample Collection and QC Sample Collection Protocol, Attachment B: ENVIRONMENTAL SAMPLING GUIDELINES AND ANALYTICAL APPROACH FOR BIOLOGICAL RESPONSE PLANS

17. Optimization of Sampling Plan (Maximizing Data Quality While Minimizing Time and Cost)

[Describe what choices were made to reduce cost of sampling while meeting the needed level of data quality. Example: The XRF will be used in situ whenever possible to achieve accurate results. Reproducibility and accuracy of in situ XRF analyses will be checked by collecting, air drying, analyzing and comparing five in situ samples at the start of sampling. Where interferences are suspected, steps will be taken to eliminate the interferences by mechanisms such as drying, grinding or sieving the samples or analyzing them using the Lumex with soil attachment.]

The format for sample number identification is summarized in Table 1. Sample collection and analysis information is summarized in Table 2.

Table 1 SAMPLE CODING		
Project Name: ____BOTE Field Exercise______ **Site ID:** _10ZZ_____		
SAMPLE NUMBER **(1)**		
Digits	**Description**	**Code (Example)**
1,2,3,4	Year and Month Code	1109
5,6,7,8	Consecutive Sample Number (grouped by SA as appropriate)	0001 (First sample of SA)

SAMPLE NAME / LOCATION ID **(2)** ***(Optional)***		
1,2,3	Floor and Room number	i.e. 101, 201
4,5	Matrix Code	AR – Air PR – Product QC – Quality Control SB - Swab WP – Wipe WT – Water
6,7	Consecutive number for each area	01

Notes:
(1) The Sample Number is a unique, 8-digit number assigned to each sample.
(2) The Sample Name or Location ID is an optional identifier that can be used to further describe each sample or sample location.

Table 2. Sampling and Analysis

Data Quality	Sampling Area	Matrix	Sampling Pattern	Sample Type	Data Quality	Number of Field Samples	Analyte or Parameter	Method Number	Action Level	Method Quant. Limit	#/type of Sample Containers per Sample	Preservative	Hold Time	Field QC
Lab Analysis	All Decision Areas	Product Wipe Swab Air	Targeted	Grab Composite	Definitive	TBD	TBD	TBD	TBD	TBD	TBD	TBD	TBD	Duplicate Blank

Note: For matrix spike and/or duplicate samples, no extra volume is required for air (unless co-located samples are collected), oil, product, or soil samples except soil VOC or NWTPH-Gx samples (triple volume). Triple volume is also required for organic water samples (double volume for inorganic).

Table 3. Common Sample Handling Information

Analysis Type	Sub Analysis	Matrix	Analytical Method	Container Type	Minimum Volume	Preservative	Temperature/ Storage	Hold Time	Source
Metals	Metals Not including Mercury or Hexachrome. Includes TAL, PP, RCRA lists)	Solid	EPA 6000 / 7000 Series	Glass Jar	200 g	n/a	None	6 months	SW-846 ch. 3
		Aqueous	EPA 6000 / 7000 Series	PTFE or HDPE	600 mL	HNO_3 to pH < 2	Not listed	6 months	SW-846 ch. 3
	Mercury	Solid	EPA 7471B	Glass Jar	200 g	n/a	$\leq 6^{o}$ C	28 days	SW-846 ch. 3
		Aqueous	EPA 7470A	PTFE or HDPE	400 mL	HNO_3 to pH < 2	Not listed	28 days	SW-846 ch. 3
	Hexavalent Chromium, (Hexachrome, Cr+6)	Solid	Lab-specific soil extraction modification, EPA 7196A	Glass Jar	100 g	n/a	$\leq 6^{o}$ C	28 days to extraction	SW-846 ch. 3
		Aqueous	EPA 218.6 (Drinking Water)	PTFE or HDPE	400 mL	n/a	$\leq 6^{o}$ C	24 hours	SW-846 ch. 3
	XRF	Solid (in situ; on the ground surface)	6200	none	n/a	none	none	Analyze Immediately	n/a
		Solid (ex situ)	6200	plastic bag	200 g	none	none	6 months	n/a
VOCs	VOCs / BTEX	Solid	EPA 5035 / 8260B	*	*	*	*	2 days to lab / 14 days	SW-846 ch. 4
		Aqueous	EPA 8260B	Amber Vial with Septa Lid	2 x 40 mL	HCl to pH< 2	$\leq 6^{o}$ C (headspace free)	14 days	SW-846 ch. 4
SVOCs	SVOCs / PAHs	Solid	EPA 8270D	Glass Jar	8 ounces	n/a	$\leq 6^{o}$ C	14 days	SW-846 ch. 4
		Aqueous	EPA 8270D	Amber Glass	2 x 1 L	n/a	$\leq 6^{o}$ C	7 days	SW-846 ch. 4
PCBs and Dioxins/Furans	PCBs	Solid	EPA 8082	Glass Jar	8 ounces	n/a	$\leq 6^{o}$ C	none	SW-846 ch. 4
		Aqueous	EPA 8082	Amber Glass	2 x 1 L	n/a	$\leq 6^{o}$ C	none	SW-846 ch. 4
	Dioxins/Furans	Solid	EPA 8280 or 8290	Glass Jar	8 ounces	n/a	$\leq 6^{o}$ C	none	SW-846 ch. 4
		Aqueous	EPA 8280 or 8290	Amber Glass	2 x 1 L	n/a	$\leq 6^{o}$ C	none	SW-846 ch. 4
Pesticides and Herbicides	Chlorinated Pesticides	Solid	EPA 8081	Glass Jar	8 ounces	n/a	$\leq 6^{o}$ C	14 days	SW-846 ch. 4
		Aqueous	EPA 8081	Amber Glass	2 x 1 L	n/a	$\leq 6^{o}$ C	7 days	SW-846 ch. 4
	Chlorinated Herbicides	Solid	EPA 8151	Glass Jar	8 ounces	n/a	$\leq 6^{o}$ C	14 days	SW-846 ch. 4
		Aqueous	EPA 8151	Amber Glass	2 x 1 L	n/a	$\leq 6^{o}$ C	7 days	SW-846 ch. 4
NWTPH	Gasoline-Range Organics	Solid	TPHs/NWTPH-Gx	Amber Glass Jar with Septa Lid	4 ounces	n/a	$\leq 6^{o}$ C (headspace free)	14 days	Method

Analysis Type	Sub Analysis	Matrix	Analytical Method	Container Type	Minimum Volume	Preservative	Temperature/ Storage	Hold Time	Source
		Aqueous	TPHs/NWTPH-Gx	Amber Vial with Septa Lid	2 x 40 mL	pH < 2 with HCl	$\leq 6^{o}$ C (headspace free)	7 days unpreserved 14 days preserved	Method
	Diesel-Range Organics	Solid	3510, 3540/3550, 8000	Glass Jar	8 ounces	n/a	$\leq 6^{o}$ C	14 days	Method
		Aqueous	3510, 3540/3550, 8000	Glass Amber	2 x 1 L	pH < 2 with HCl	$\leq 6^{o}$ C	7 days unpreserved 14 days preserved	Method
Geotechnical	Particle Size Analysis	Solid	ASTM D-422	Glass Jar or Plastic Bag	2 x 8 ounce	none	n/a	n/a	Method
Miscellaneous	pH	Solid	EPA 9045	Glass Jar	8 ounces	n/a	n/a	Analyze Immediately	SW-846 ch. 3
		Aqueous	EPA 9040	PTFE	25 mL	n/a	n/a	Analyze Immediately	SW-846 ch. 3
	Total Organic Carbon (TOC)	Solid	SW-846 9060	Glass Jar	100 mL	n/a	$\leq 6^{o}$ C	28 days	SW-846
		Aqueous	EPA 415.1	PTFE or HDPE	200 mL	store in dark HCL or H_2SO_4 to pH <2	$\leq 6^{o}$ C	7 days unpreserved 28 days preserved	Method
	Cyanide	Solid	SW-846 9013	Glass Jar	5 g	n/a	$\leq 6^{o}$ C	14 days	SW-846 ch. 3
		Aqueous	SW-846 9010C	PTFE or HDPE	500 mL	NaOH to pH > 12	$\leq 6^{o}$ C	14 days	SW-846 ch. 3
	Conductivity	Aqueous	EPA 120.1	PTFE or HDPE	100 mL	n/a	n/a	Analyze Immediately	Method
	Hardness	Aqueous	EPA 130.1	PTFE or HDPE	1 x 1 L	HNO3 to pH<2	$\leq 6^{o}$ C	28 days	Method
	Total Suspended Solids	Aqueous	EPA 160.2	PTFE or HDPE	100 mL	n/a	$\leq 6^{o}$ C	7 days	Method
	Total Dissolved Solids	Aqueous	EPA 160.1	PTFE or HDPE	100 mL	n/a	$\leq 6^{o}$ C	7 days	Method
	Nitrate/nitrite	Aqueous	EPA 353.2	PTFE or HDPE	1 x 250 mL	H_2SO_4 to pH <2	$\leq 6^{o}$ C	28 days	Method
	Nitrate	Aqueous	SW-846 9210A	PTFE or HDPE	1,000 mL	n/a	$\leq 6^{o}$ C	28 days	SW-846 ch. 3
	Nitrite	Aqueous	SW-846 9216	PTFE or HDPE	25 mL	n/a	$\leq 6^{o}$ C	48 hours	SW-846 ch. 3, Method
	Fluoride	Aqueous	SW-846 9214	PTFE or HDPE	300 mL	n/a	$\leq 6^{o}$ C	28 days	SW-846 ch. 3
	Chloride	Aqueous	SW-846 9250	PTFE or HDPE	50 mL	n/a	$\leq 6^{o}$ C	28 days	SW-846 ch. 3
	Sulfate	Aqueous	SW-846 9035	PTFE or HDPE	50 mL	n/a	$\leq 6^{o}$ C	28 days	SW-846 ch. 3
	Sulfide	Solid	SW-846 9215	Glass Jar	1 x 4 ounces	Fill sample surface with 2N zinc acetate until moistened.	$\leq 6^{o}$ C (headspace free)	7 days	SW-846 ch. 3
		Aqueous	SW-846 9031	PTFE or HDPE	100 mL	4 drops 2N zinc acetate/100 mL sample; NaOH to pH>9.	$\leq 6^{o}$ C (headspace free)	7 days	SW-846 ch. 3

Key:

*	= See individual methods. We typically collect 3xEnCore-type samplers and 1x40 mL VOA vial per sample, keep at $\leq 6^{o}$C with no chemical preservative, and they must be at the lab within 48 hours of collection.				
C	= Celsius	HNO_3	= nitric acid	SVOCs	= semivolatile organic compounds

Cr	= chromium	L	= liter	SW-846	= EPA Test Methods for Evaluating Solid Waste, Physical/Chemical Methods
EPA	= Environmental Protection Agency	mL	= milliliter	TAL	= Target Analyte List
g	=grams	n/a	= not applicable	TPH	= total petroleum hydrocarbons
H2SO4	= sulfuric acid	NaOH	= sodium hydroxide	VOA	= Volatile Organic Analysis
HCL	= hydrochloric acid	PCBs	= polychlorinated biphenyls	VOCs	= Volatile Organic Compounds
HDPE	= high-density polyethylene	PTFE	= polytetrafluoroethylene		
Hg	= mercury	RCRA	= Resource Conservation and Recovery Act		

III. Assessment and Response

A Sample Plan Alteration Form (SPAF) will be used to describe project discrepancies (if any) that occur between planned project activities listed in the final SSSP and actual project work. The completed SPAF will be approved by the OSC and QAC and appended to the original SSSP.

A Field Sampling Form (FSF) may be used to capture the sampling and analysis scheme for emergency responses in the field and then the FSF pages can be inserted into the appropriate areas of the final SSSP.

Corrective actions will be assessed by the sampling team and others involved in the sampling and a corrective action report describing the problem, solution, and recommendations will be forwarded to the OSC and the ERU QAC.

IV. Data Validation and Usability

The sample collection data will be entered into Scribe and Scribe will be used to print lab Chains of Custody. Results of field and lab analyses will be entered into Scribe as they are received and uploaded to Scibe.net when the sampling and analysis has been completed.

18. Data Validation or Verification will be performed by:

ERU's general recommendation on validation is that a minimum of CLP-equivalent stage IIA verification and validation be performed for every SSSP involving laboratory analyses. However, stage IIB is preferred if the lab can provide it. Dioxins should be validated at CLP-equivalent stage 4.

	Data Verification and Validation Stages						
Performed by:	I	IIA	IIB	III	IV	Verification	Other:
Contractor QA Reviewer							
Contractor QA Reviewer							
EPA QA Office							
MEL staff							
Other:Unified Command			100%			100%	